Determinants of sphincter preservation in low rectal surgery for cancer

A thesis submitted in partial fulfilment of the requirements of the Degree of Doctor of Medicine (Research)

January 2020

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Statement of originality

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Abstract

Introduction

The assessment and management of rectal cancer is complex, involving clinicians from several specialities, with a broad and expanding range of therapeutic options. As oncological outcomes have improved, the preservation of functioning sphincters with avoidance of long-term functional problems, and the negative impact that these have on quality of life, is a priority in the management of rectal cancer.

Aims and objectives

This thesis aimed to discover what determines the ability to preserve functioning sphincters during the management of low rectal cancer.

Objectives were (1) to systematically review the evidence base for treatment of rectal cancer (2) to determine the proportion of patients following anterior resection in the UK with low anterior resection syndrome (LARS) and identify risk factors (3) to determine ability of measures derived from diffusion-weighted MRI (DWI) to predict and assess response to neoadjuvant chemoradiotherapy (CRT) in rectal cancer (4) to identify microRNA targets with potential as predictive biomarkers of response to CRT and (5) to define altered sphincter function following anterior resection and CRT using high-resolution anorectal manometry (HRAM).

Methods

Objective 1: A systematic review of the current literature. Objective 2: A prospective epidemiological cohort study using LARS and QLQ-C30 quality of life questionnaires. Objective 3: A retrospective cohort study of patients undergoing DWI for rectal cancer. Objective 4: A retrospective MicroRNA profiling using pre-treatment rectal cancer biopsies. Objective 5: A prospective cohort study of rectal cancer patients undergoing HRAM.

Results

A review of the literature showed that there is data on oncological outcomes following sphincter preserving rectal cancer surgery, but data on functional

outcomes is limited. 1093 participants completed the LARS questionnaire, 22% had minor LARS and 41% major LARS. The risk factors for LARS identified by the study were neoadjuvant radiotherapy, defunctioning stoma, female gender and younger age. The DWI study included 39 patients and found that use of DWI was feasible and that measures derived from DWI show potential as non-invasive biomarkers for predicting and assessing response to CRT in rectal cancer. The laboratory study confirmed that analysis of microRNA expression from rectal biopsy tissue from 28 patients was feasible and that, with further study, microRNAs could possibly act as predictive biomarkers for response to neoadjuvant therapy. 51 patients underwent HRAM, which showed potential for being a tool to help improve assessment and understanding of pre and post treatment sphincter function in patients with rectal cancer.

Conclusions

Patients undergoing sphincter-preserving surgery for rectal cancer are at risk of developing LARS. This has a long-term impact on quality of life. Neoadjuvant therapy significantly increases risk of LARS. Imaging and laboratory studies confirmed that DWI and microRNA analysis are feasible approaches to the identification of potential biomarkers; with further study these could meet the aim of individualised therapy and limit use of neoadjuvant therapy to preserve anorectal function where possible. The exact pathophysiology behind LARS remains unexplained; HRAM can be used as an investigative tool to improve this understanding with the aim to preserve function. Multicentre prospective studies incorporating all of these methods are required.

Acknowledgements

I would like to acknowledge my supervisors, Mr Mohamed Thaha and Professor Andy Silver.

The David Johnston Fellowship from the Royal College of Surgeons of England funded this MD and the LARS questionnaire study. I am very grateful to both Professor David Johnston himself, who personally funded the Fellowship, as well as to Professor Sir Norman Williams, who was instrumental in this process and provided personal support during the first few years.

Professor Harry Staines provided statistical support, patiently crosschecking my analyses, and his advice, based on his extensive experience of interpreting clinical research, has been invaluable. Professor Charlie Knowles has helped me personally and practically at several key points, I have really enjoyed working with him. Mr Christopher Chan gave crucial advice at the early stages of this thesis, which led it to develop into its current shape.

Surgeons, nurses and MDT coordinators from all over the UK participated in the LARS questionnaire study and I am very grateful for their enthusiasm and dedication to this important area of research. In particular, I would like to thank Professor Bob Steele, Dr Duncan Gilbert and Dr Claire Taylor. I am also grateful to those who provided support with the practicalities of setting up and running a study across the UK, including Emma Horrocks, Natasha Stevens, Ellie Scott, Ugo Grossi and Sybil Bannister. This thesis would not have been possible without the patients who completed the questionnaire and in many cases also wrote, called or emailed, taking time to explain their experiences and their hopes for the future. They inspired me to complete this work and continue to inspire me to always focus on quality of life.

A number of clinicians at Barts Health NHS Trust provided help and guidance during this MD. Mr Shafi Ahmed and Dr Amen Sibtain both gave useful advice. I would like to thank Dr Arman Parsai and Dr Marc Miquel for their patience in teaching MRI interpretation and the physics behind diffusion-weighted imaging. Professor Roger Feakins kindly gave up his time to reassess tissue regression grades for chapters 3 & 4. He also gave very useful advice about the histopathological variables to include in both studies. Dr Mark Scott introduced me to the world of

high resolution manometry and provided support throughout this MD. He also provided Figures 5.3 and 5.4 for chapter 5. I am also grateful to Dr Emma Carrington for her advice and help with definitions in chapter 5. All of the staff in the GI Physiology Unit were friendly, helpful and supportive and brightened many long days.

Without the endless patience of Professor Andy Silver and his team, I would not have been able to carry out the lab work needed for chapter 4. Andy taught me a new language, culture and skillset and I will miss our long chats about what to do next. Dr Nirosha Suraweera, Dr Anke Nijhuis, Dr Amy Lewis and Dr Hannah Thompson provided practical teaching in lab skills and help interpreting results. Dr Anke Nijhuis also kindly provided hypoxia samples for chapter 4. Rosemary Jeffery was very helpful in locating slides and specimens. I am also grateful to Dr Sham Mehta who gave specific, and sometimes entertaining advice, about juggling clinical work, lab work and childcare.

My friends have been an important source of relief and a much needed distraction at times. My family have cheered me on and kept me sane all the way, particularly my Mum, Sally, Matt, Lily, Julia and Scott.

More than anyone else, this MD has only been possible with the support of my husband, Adipat Virdi. His love, kindness, enthusiasm and practical help with every aspect of my work and my life have enabled me to complete this thesis. His questioning about every finding and every conclusion, have made me challenge my own ideas and assumptions, and inspired me to explain myself clearly and to be a better writer. Adi has also looked after our son, Kip, giving me the space and time to work.

This thesis is dedicated to Kip, who has shared his Mumma with it for his entire life..x..

Contents

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List of abbreviations

5-FU	5-Fluorouracil
ACPGBI	Association of Coloproctologists of Great Britain and Ireland
ADC	Apparent Diffusion Coefficient
AJCC	American Joint Committee on Cancer
APER	Abdominoperineal Excision of Rectum
ARS	Anterior Resection Syndrome
AUC	Area Under the Curve
CCCS	Cleveland Clinic Constipation Score
cCR	Clinical Complete Response
cDNA	Complementary DNA
CEA	Carcinoembryonic antigen
CI	Confidence Intervals
CIN	Chromosomal Instability
COX-2	Cyclooxygenase-2
CPEX	Cardio-Pulmonary Exercise Testing
CR	Complete Response
CRC	Colorectal Cancer
CRM	Circumferential Resection Margin
CRT	Chemoradiotherapy
DNA	Deoxyribonucleic Acid,
DRE	Digital Rectal Examination
DWI	Diffusion-weighted MRI
EGFR	Epidermal Growth Factor Receptor
ELAPE	Extralevator Abdominoperineal Excision of Rectum
EMR	Endoscopic Mucosal Resection
EMVI	Extramural Venous Invasion
EORTC	European Organization for Research and Treatment of Cancer
EPI	Echoplanar Imaging
ERUS	Endo-Rectal Ultrasound
FACL	Functional Anal Canal Length
FACT	Functional Assessment of Cancer Therapy
FAP	Familial Adenomatous Polyposis
FFPE	Formalin Fixed Paraffin Embedded
FOV	Field-Of-View
Gy	Grays

H&E	Haematoxylin and Eosin
HNPCC	Hereditary Non-Polyposis Colon Cancer
IQR	Interquartile Range
LARS	Low Anterior Resection Syndrome
LCRT	Long Course Chemoradiotherapy
MACS	Microsatellite and Chromosome Stable
MAP	MYH-Associated Polyposis
MDT	Multidisciplinary Team
MRI	Magnetic Resonance Imaging
NBCA	National Bowel Cancer Audit
NICE	National Institute for Health and Care Excellence
NPV	Negative Predictive Value
NRES	National Research Ethics Service
NSABP	National Surgical Adjuvant Breast and Bowel Project
OR	Odds Ratios
pCR	Pathological Complete Response
PET-CT	Positron Emission Tomography-Computed Tomography
PIC	Participant Identification Centre
PME	Partial Mesorectal Excision
PPV	Positive Predictive Value
PROMs	Patient Reported Outcome Measures
QOL	Quality of Life
qPCR	Quantitative Polymerase Chain Reaction
RNA	Ribonucleic Acid
ROC	Receiver-Operating Characteristic
ROI	Regions of Interest
SCRT	Short Course Radiotherapy
SE	Spin Echo
SMIS	St Marks Incontinence Score
SPSS	Statistical Package for the Social Sciences
TEM	Transanal Endoscopic Microsurgery
TME	Total Mesorectal Excision
TRG	Tumour Regression Grade
TSE	Turbo Spin Echo
UC	Ulcerative Colitis
UICC	Union for International Cancer Control
VEGF	Vascular Endothelial Growth Factor

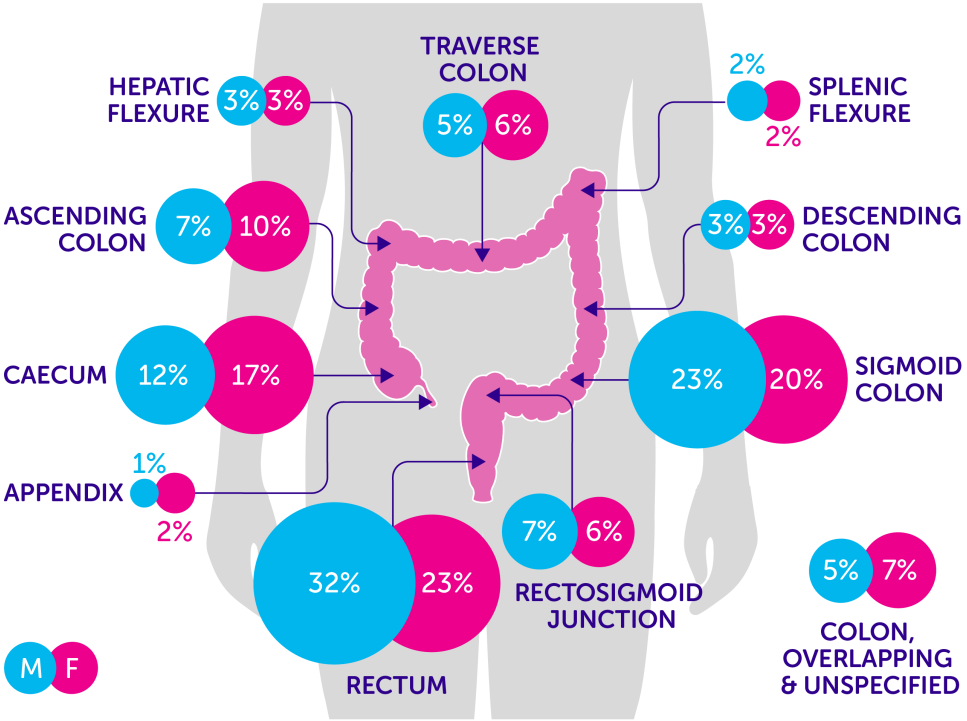
Chapter 1. Introduction

1.1 Epidemiology

1.1.1 Epidemiology of rectal cancer

Colorectal cancer (CRC) is the fourth most common cancer in the UK behind lung, breast and prostate, and is the third most common cancer in both men and women[1]. Incidence is linked strongly to age; with 94% of cases occurring in people aged over 50[1]. Even though incidence is stable, overall 5-year survival rates continue to improve, having almost reached 55%. Despite this, CRC remains the second biggest cause of cancer deaths in the UK. Rectal cancer makes up around 28% of CRCs, with a further 7% at the rectosigmoid junction[1]; Figure 1.1 shows the distribution of cancers throughout the colon. The incidence of rectal cancer is 23 per 100,000 people per year in the UK, this translates into over 14,000 new cases of rectal cancer annually and incidence of rectal cancer is higher in men, making up over 60% of cases[1].

Figure 1.1 Percentage distribution of bowel cancer cases in the UK 2010-2012, for men and women[1].



1.1.2 Epidemiology of sphincter preservation

Several developments in recent years have facilitated a move towards sphincter-preserving surgery for low rectal cancer. These include: an appreciation of the need for total mesorectal excision; technological improvements in stapling equipment and an improved understanding of pathology, leading to gradual reductions in the minimum acceptable distal margin[2,3]. Despite the resulting fall in rates of abdominoperineal excision of the rectum (APER), 20-30% of patients undergoing surgery for rectal cancer are still left with a permanent stoma[4].

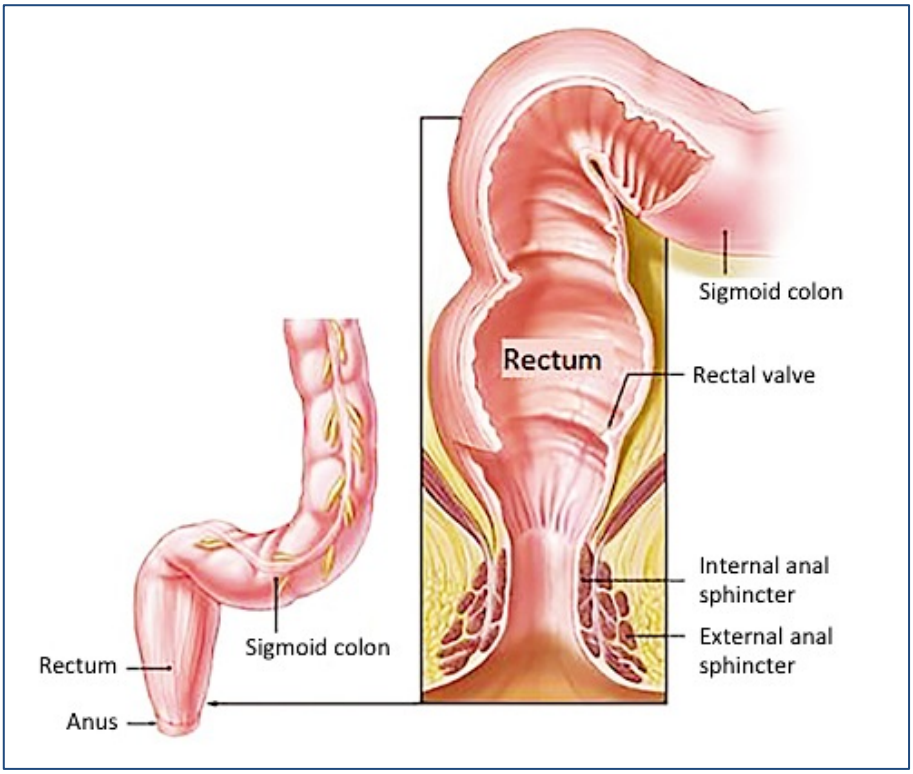
In the 2018 National Bowel Cancer Audit (NBCA) report, 26% of patients undergoing a major resection for rectal cancer had an APER with formation of a permanent stoma[5]. Sphincter-preserving procedures were more common with 9% having a Hartmann's procedure and 63% an anterior resection[5]. However, even a proportion of patients undergoing sphincter-preserving surgery may still be left with a stoma. A study looking at the reversal rates following Hartmann's procedure in England has shown that for patients who undergo this procedure for cancer, only 8.3% are subsequently reversed[6]. The NBCA report shows that 77% of patients having an anterior resection had a temporary ileostomy created[5]. At 18 months, 28% of those having an anterior resection still had a stoma[5]. This reflects research findings that show that up to 25% of these 'temporary' stomas are never reversed[7]. Overall, of patients undergoing a major resection for rectal cancer, 52% had a stoma at 18 months. Rates of stoma at 18-months were highly variable across the UK NHS Trusts, ranging from between 42-63%[5].

1.2 Applied rectal anatomy

The rectum is the most distal part of the colon and is usually 12 to 15cm in length. The rectum begins at the rectosigmoid junction where the taenia coli of the colon coalesce from 3 distinct bands into a single longitudinal muscle layer[8]. The rectum is mostly extraperitoneal although anteriorly a thin layer of visceral peritoneum covers the front and sides of the rectum down to the peritoneal reflection[8]. Endoscopically, the most important landmarks are the rectal valves (the circular valves of Houston), these are rectal folds that can be variable in position but roughly divide the rectum into upper, middle and lower thirds[9] (see Figure 1.2). The rectum dilates slightly below the middle fold and this area is known as the rectal ampulla[10]. Other

landmarks that have been used to define the rectum include the level of the sacral promontory or the peritoneal reflection but these can be variable[11]. Rectal length can also be variable and is affected by several factors including body size, pelvimetry and gender[10].

Figure 1.2 Rectal anatomy (adapted from[12])



The distal limit of the rectum can be defined as either the muscular anorectal ring or the dentate line[13]. The dentate line marks an important transition point, partly because it is visible but also because it delineates the point at which the blood supply and innervation change to those of the anal canal[9]. The most distal part of the rectum lies within the pelvic floor musculature; this section of the rectum, which varies considerably in length, has been termed the rectal 'no man's land' and can be difficult to resect via an abdominal approach[14].

Distal to the rectum, the anal canal extends from the anorectal junction to the anal verge. The anal canal also varies in length. The anal sphincter is formed from 2 muscles, the internal and external anal sphincters. The internal sphincter is made up of smooth muscle and is the continuation of the inner circular smooth muscle layer of the rectum. The external muscle is skeletal and although it is in a constant state of tonic contraction, it is also under voluntary control[9].

1.3 Definitions used in rectal cancer surgery

1.3.1 Rectal cancer

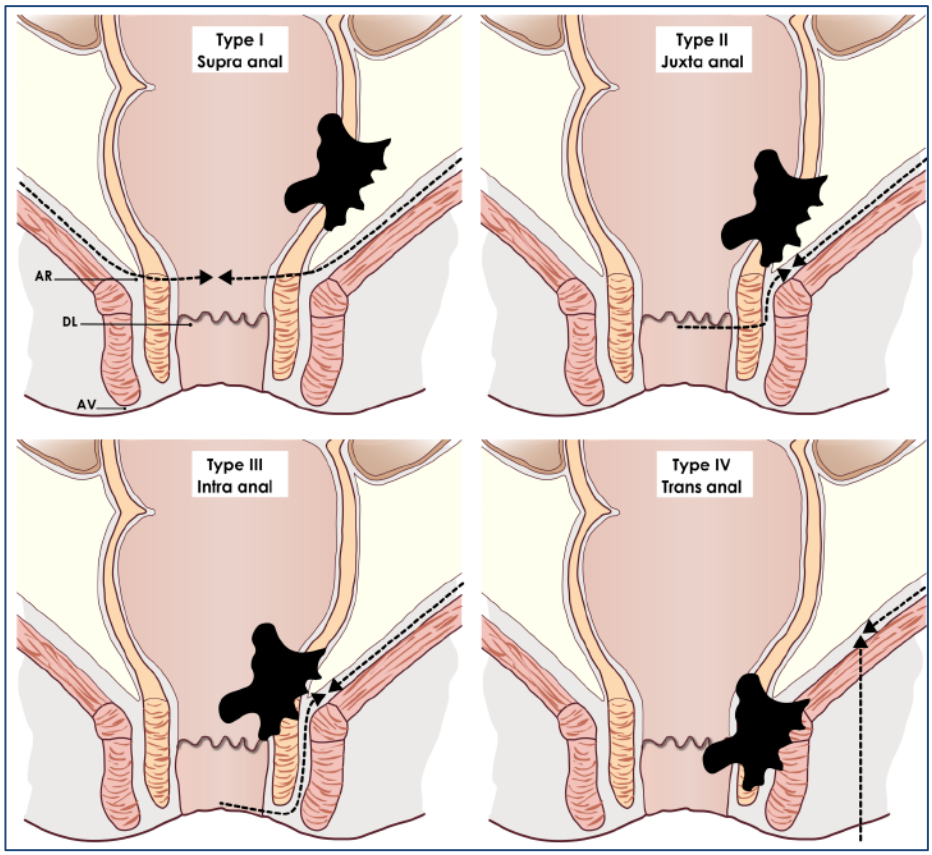
Rectal cancer can be classified in several different ways, including high vs. low, early vs. late and 'locally advanced'. Due to the variable definitions of the rectum itself, there is also variation in what is defined as a rectal cancer. A recent review of National and International guidelines on rectal cancer found that four guidelines described this as 'any cancer within 15cm of the anal verge', and two used '12cm from the anal verge' as the limit. However, even the method used to measure this distance varied, with six guidelines using rigid proctoscopy and two using tumour height on MRI[15]. A study of 123 surgeons from 28 countries assessed their agreement with the different definitions given for the rectum and found that only 50% agreed with a definition of '15cm from the anal verge', with others preferring measurement from the dentate line[16].

1.3.2 Low rectal cancer

As described above, the rectum can be divided into three parts, with the low rectum defined as 0-6cm from the anal verge, the mid rectum 7-11cm and the upper rectum 12-15cm[10]. This certainly corresponds with the definition of low rectal cancer used by LOREC – the Low Rectal Cancer Development programme, which is: "an MRI-based anatomical definition where the mesorectum tapers at the origin of the levators, at the pelvic sidewall. This usually corresponds to a measurement of within 6 centimetres of the anal verge"[17].

Rullier *et al.* have proposed a classification of low rectal tumours into 4 groups based on the location of the tumour in relation to the anal sphincter (illustrated in Figure 1.3)[18]. They advise different treatment strategies for tumours in each group. This classification avoids some of the issues with definition outlined above, as by referencing the tumour to the sphincter mechanism, problems with variation in anal canal length are avoided[18]. This system has also been shown, by the same authors, to be identifiable and reproducible on MRI[19].

Figure 1.3 The classification of low rectal tumours proposed by Rullier and colleagues. AR: anal ring; DL: dentate line; AV: anal verge. The dotted line indicates the plane of surgical dissection required[18].



1.3.3 Early rectal cancer

Early rectal cancer has been defined as, "invasive adenocarcinoma, spreading into, but not beyond, the submucosa, that is a T1 tumour in the tumour node metastasis (TNM) classification"[20]. However, others have included T2N0 tumours within the definition of early rectal cancer[21]. The issue with using TNM staging within the definition is that this information is only available following histological excision and is not useful when deciding which tumours may be locally excised. Because of this, the European Association for Endoscopic Surgery have recently defined early rectal cancer as, "a rectal cancer with good prognostic features that might be safely removed preserving the rectum and that will have a very limited risk of relapse after local excision"[22].

1.3.4 Locally advanced rectal cancer

The EURECA-CC2 European Rectal Cancer Consensus Conference defined locally advanced tumours as, "neoplasms extending beyond the rectal wall with unresectable infiltration to surrounding organs or structures, and/or perforation of the visceral peritoneum (T4 N0–2 M0)"[23]. However, the term is more widely used, particularly in a research setting, to include all stage II tumours (T3 or T4) and all stage III tumours (node positive), even by authors who participated in the consensus conference[24]. The proportion of tumours referred to as locally advanced is increasing and confusion over which tumours are classified by this term hinders discussions on treatment[25].

1.4 Clinical assessment of rectal cancer

1.4.1 Presentation

The most common presenting symptoms of rectal cancer include: rectal bleeding; tenesmus; change in bowel habit; weight loss and symptoms of anaemia. The majority of patients presenting with rectal cancer are referred by their GP and seen in the outpatient clinic within 2 weeks according to NHS guidelines[26]. 20% of patients with CRC present as an emergency and for those undergoing emergency surgery, prognosis is much worse than for elective patients[5]. The NHS Bowel Screening Programme was introduced in 2006, screening patients every 2 years from the ages of 60-74 using faecal occult blood testing[27]. Between 2006 and 2010, 5% of patients undergoing resection for CRC presented via this route[26], this proportion had increased to 10% in the most recent NBCA report[5].

1.4.2 Diagnosis

The use of digital rectal examination (DRE) depends on the height of the tumour. In palpable tumours it can be used to determine fixation of the tumour, sphincter involvement, location within the rectum and an accurate assessment of tumour height[28]. It has limited use for the determination of depth of invasion, showing only 65% agreement with histological assessment[29]. This study also demonstrated the limitations of DRE for assessing rectal cancers as 24% of patients had tumours that were too high or found examination too painful. A further study did find that

identification of a non-fixed tumour on DRE was a significant predictor of good response to neoadjuvant chemoradiotherapy (CRT)[30]. Rigid sigmoidoscopy is often used in an outpatient setting to confirm the precise location of the tumour within the rectum and assess tumour height from the anal verge. This has been shown to produce significantly different measurements to those on MRI, which is partly due to the straightening of the rectum that occurs during sigmoidoscopy[31].

Routine blood tests are usually done to assess general fitness and in preparation for treatment. Carcinoembryonic antigen (CEA) is used as a tumour marker in CRC. It is detectable in serum and is used during diagnosis, monitoring treatment effect and in follow-up. Use of CEA in the detection of CRC is stage-dependent, with CEA > 2.5ng/ml having a sensitivity of 36% and specificity of 87% for stage I and II cancers, a sensitivity of 74% for stage III and 83% for stage IV tumours[32]. CEA can be raised in smokers, in inflammatory conditions and in a range of other cancers; these issues in combination with the low sensitivity in early CRC make it an unsuitable test for population screening[33]. A study of 98 patients undergoing neoadjuvant CRT for rectal cancer showed that pre-treatment CEA <3.0ng/ml was a significant predictor of good pathological response to treatment[30].

Colonoscopy allows direct inspection of the mucosal surface of the large bowel and is usually the first line investigation for all patients with suspected CRC. The location of the tumour can be assessed and biopsies taken to allow histological diagnosis. This also allows for identification of any polyps or synchronous lesions in the remainder of the colon. In patients who are less fit to undergo this invasive test, they may undergo virtual colonoscopy using 3D reconstruction of images taken with CT. This has good specificity and sensitivity for polyps and tumours over 1cm in size but is less accurate for smaller lesions and does not permit biopsy[34].

1.4.3 Staging

The aim of the staging investigations is to determine the location and extent of the tumour, along with nodal, vascular or neural infiltration. This allows classification of the patient into a low, medium or high risk category, which in turn determines which treatment route will be followed[35].

Staging of rectal cancer is classified according to the TNM system as described by the American Joint Committee on Cancer[36]. This internationally recognised system

allows reproducible and consistent staging. Tumours can also be grouped into stages I to IV (as shown in Table 1.1), which predicts prognosis and dictates management strategies. Dukes' staging was traditionally used in the UK prior to the introduction of the AJCC system and is occasionally referred to; it fits with AJCC staging as shown in Table 1.1 below[37]. Some variables, which are used in decision-making regarding neoadjuvant therapy, and have a bearing on outcomes in rectal cancer, are not currently included in the TNM system, most importantly involvement of the circumferential resection margin (CRM) and vascular invasion.

Table 1.1 TNM staging of rectal cancer. Taken from AJCC 7th ed[36].

Primary Tumour (T)				
T0	No evidence of primary tumour			
T1	Tumor invades submucosa			
T2	Tumor invades muscularis propria			
T3	Tumor invades through the muscularis propria into pericolorectal tissues			
T4a	Tumor penetrates to the surface of the visceral peritoneum			
T4b	Tumor directly invades or is adherent to other organs or structures			
Regional Lymph Nodes (N)				
N0	No regional lymph node metastasis			
N1	Metastasis in 1-3 regional lymph nodes			
N2	Metastasis in 4 or more regional lymph nodes			
Distant Metastasis (M)				
M0	No distant metastasis			
M1	Distant metastasis			
Stage Grouping				
	T	N	M	Dukes staging
Stage I	T1 - T2	N0	M0	A
Stage II	T3 - T4b	N0	M0	B
Stage III	T1 - T4b	N1 - N2	M0	C
Stage IV	Any T	Any N	M1	-

1.4.4 Staging investigations

Magnetic resonance imaging (MRI) is the gold standard for local staging in rectal cancer in the UK and facilitates surgical planning and selection of patients for

neoadjuvant therapy. Guidelines for the Management of Cancer of the Colon, Rectum and Anus from the Association of Coloproctologists of Great Britain and Ireland (ACPGBI) and Guidelines on Colorectal Cancer from the National Institute for Health and Care Excellence (NICE) state that all patients with rectal cancer should undergo MRI unless there is any contraindication to this[38,39]. The most recent NBCA data shows that 85% of patients with rectal cancer in the UK had an MRI[5]. For tumours ≥T2, MRI is the most useful imaging modality for assessing depth of invasion of the primary tumour and determining involvement of the CRM[40]. It has a shorter learning curve than other imaging modalities and findings can easily be demonstrated, making it useful for a multi-disciplinary team (MDT) setting[28]. Data from the MERCURY study group have shown the accuracy of MRI in predicting a clear CRM to be as high as 91%[41]. They have also shown that MRI is accurate to within ±0.5mm in assessing the distance of extra-mural invasion compared with histological analyses[41].

MRI staging can be used to accurately predict those who do not need CRT even with a T3 tumour. Features on MRI shown to be prognostic of good outcome include: cancer <5mm from the muscularis propria (i.e. early T3); CRM >1mm and absence of extramural vascular invasion[42,43]. Using these criteria to determine management, patients who underwent surgery without neoadjuvant therapy showed a low recurrence rate of 3%[42].

MRI is not so sensitive and specific for the assessment of nodal involvement although this is a problem that affects all imaging modalities used in rectal cancer staging[44]. A meta-analyses of imaging techniques in rectal cancer found the overall sensitivity of MRI to detect metastatic lymph nodes was 66%, with specificity of 76%[45]. Lymph node size has been identified as the most reliable parameter to predict nodal involvement on MRI[46] but there is a lack of consensus about the exact size criteria to use, and studies have used various size cut-offs from 3 – 10mm[47]. There is also considerable overlap in size between normal and metastatic nodes[48] and some studies have found nodal size to be an unreliable indicator of lymph node metastases[49]. Nodal size is certainly not the only determinant of involvement and other features, including regularity in the shape of the node and signal intensity, are also important[50]. A study assessing involvement of lymph nodes on MR showed that the presence of a spiculated or irregular border showed specificity of 100%, although sensitivity was still low at 45 and 36%[47]. The presence of a mottled heterogeneous appearance showed sensitivity of 50% and specificity of 100%[47].

Standard T2 weighted MRI imaging has some limitations in the restaging of rectal cancer following neoadjuvant therapy, it overcalls based on peri-tumoral inflammation, ulceration, fibrosis and proctitis[47]. The restaging of lymph nodes in the mesorectum is affected by similar changes[47]. In the MERCURY study data, the accuracy of CRM involvement on restaging MRI scans dropped to 77%[50]. A meta-analysis has shown the accuracy of restaging scans for predicting T stage to vary from 34 to 82% with an overall value of 52%[51]. T0 and T1 tumours were frequently overstaged[51]. Overall accuracy for restaging of nodal involvement was higher at 72%, ranging from 60 to 88% in individual studies[51]. A prospective study analysing data from 5 large prospective databases showed similar results, demonstrating poor accuracy for the prediction of N and T stage as well as inability to predict complete pathological response or discriminate T4 disease[52].

As a result of these problems in the interpretation of standard MRI images following neoadjuvant therapy, a technical evolution is occurring in MRI with the development of new modalities including diffusion-weighted MRI, perfusion MRI and lymph-node specific contrast agents[28].

Diffusion weighted imaging (DWI) MRI is a functional imaging technique, which utilises the way in which different tissues affect the dynamics of water molecule diffusion, to provide detailed information about tumours and peri-tumoral changes such as necrosis, fibrosis and inflammation[53]. Use of DWI is increasing but the evidence base is incomplete; potential benefits in terms of predicting clinical outcomes and determining response to neoadjuvant therapy are not yet fully established[53].

Endo-rectal ultrasound (ERUS) is more commonly used in the staging of rectal cancer in the USA and Europe, but less so in the UK. It is more accurate for early stages of rectal cancer, specifically the diagnosis of T1 and T2 lesions. A meta-analysis has shown ERUS to have sensitivity of 90% for assessing perirectal tissue involvement, significantly higher than that of MRI at 82%[45]. ERUS has some benefits; it can be used in an outpatient setting by a variety of health professionals to provide rapid information about a tumour. However, drawbacks include that it is user dependent with inter-observer variation of up to 15%[54] and has a longer learning curve than other modalities[55]. ERUS has poorer sensitivity and specificity for nodal assessment than for T stage, with a sensitivity of 67% and specificity of 78% on meta-analyses, comparable to results for MRI[45]. However, ERUS is unable to define the

involvement of the CRM and since that plays a major role in pre-operative planning, it is often used alongside MRI.

For restaging, ERUS shows highly variable accuracy for T stage assessment, ranging from 26 to 93% with an overall value of 65%[51]. Inaccuracy can arise due to difficulty differentiating tumour from desmoplastic reactions and peri-tumoral fibrosis[28]. Showing a similar pattern to MRI, overall accuracy for restaging of nodal involvement was higher than for T stage, at 73%[51].

Contrast-enhanced CT of the chest, abdomen and pelvis is currently used in the UK to assess distant nodal and metastatic disease. Thoracic CT is of increased importance in the staging of rectal cancer as rectal tumours spreads preferentially to the lungs not the liver due to the rectal blood supply bypassing the portal system[35]. Retrospective studies have confirmed this increased risk of lung metastases, showing a frequency of 9 to 18% in patients with rectal cancer[56,57]. CT has limited accuracy for local staging and the potential for replacing this modality with early whole body MRI, which would incorporate local and distant staging, is currently being investigated[58]. Repeat CT scanning is usually carried out as part of restaging, following neoadjuvant therapy and prior to surgery, although there are no guidelines on this. A small study assessing whether this interval CT made any difference found that a change in treatment strategy due to new findings on CT occurred in 12% of patients[59].

Positron emission tomography (PET) CT scanning is primarily used in the assessment of distal nodal involvement and metastatic disease rather than in primary staging. It cannot provide detailed anatomical information and is limited to identifying lesions over 5mm[60]. It therefore has limited use in assessing T stage. It also has a low sensitivity for detecting nodal involvement, again because many affected nodes will be smaller than 5mm and also because the effect of the primary lesion masks nearby nodes[60]. Despite this, a number of studies have shown that including PET-CT in the protocol for the staging of rectal cancer leads to upstaging or downstaging and a subsequent change of management in up to 38% of patients[61-63].

PET-CT has also been investigated for the assessment of response to neo-adjuvant therapy and ability to predict complete pathological response to therapy. A recent meta-analysis showed good accuracy for assessment of response with pooled

sensitivity of 73% and specificity of 77%[64]. Individual studies have shown even greater accuracy when data is combined from PET-CT scans before, during and after neoadjuvant treatment[65]. Results from a prospective trial of observation following complete clinical response to neoadjuvant therapy show that PET-CT findings compared with clinical and pathological findings resulted in a sensitivity of 93% and specificity of 53% to predict complete response[66]. The advantages and disadvantages of ERUS, MRI and PET-CT are summarised in Table 1.2.

Table 1.2 Advantages and disadvantages of different imaging techniques for staging and restaging of rectal cancer ERUS: Endo-rectal ultrasound; MRI: magnetic resonance imaging; PET-CT: Positron emission tomography-computed tomography; CRM: circumferential resection margin (adapted from[28]).

	Staging		**Restaging**	
	Pros	Cons	Pros	Cons
ERUS	High accuracy and specificity for early rectal cancer	Poor N staging Operator dependent Learning curve	High accuracy for persistent nodal involvement	Low accuracy for T restaging
MRI	CRM evaluation High accuracy in advanced tumours Best tool for selecting patients for neoadjuvant therapy	Low accuracy for lymph node involvement	Good prediction of CRM involvement	Poor accuracy in predicting T0 and N0
PET-CT	Confirmation of M and N at distant sites	Low accuracy for T staging	Detection of progression at distant sites	Lack of standardisation in assessing response

1.5 Multi-disciplinary team approach to rectal cancer management

The NICE and ACPGBI Guidelines[38,39] advise that all patients with CRC should be discussed at an MDT. This process was introduced following two reports, the Calman Hine report[67] and Guidance on Commissioning Cancer Services[68]. These two

documents led to significant changes in delivery of care, from an individual approach, to a multi-disciplinary team (MDT) based approach. The ACPGBI guidelines also specify the core members of an MDT; these recommendations are shown in Box 1.1[38]. The guidelines also recommend that the core MDT team should have regular liaison with surgeons from a liver MDT and thoracic MDT as required[38].

Box 1.1 Core members of the multi-disciplinary team from the ACPGBI Guidelines for the Management of Cancer of the Colon, Rectum and Anus. MDT: multi-disciplinary team[38].

Core members of the MDT team:

- Specialist surgeons (at least 2)
- Clinical oncologist
- Medical oncologist
- Diagnostic radiologist with gastrointestinal expertise
- Histopathologist
- Colonoscopist (surgeon, physician or specialist nurse)
- Clinical nurse specialist (CNS)
- Clinical trials co-ordinator or research nurse
- Palliative care specialist (doctor or nurse)
- MDT co-ordinator
- Administrative support (including data manager)

The extended MDT members should include:

- Gastroenterologist
- Liver surgeon
- Thoracic surgeon
- Interventional radiologist
- Dietician
- Liaison psychiatrist/clinical psychologist
- Social worker
- Clinical geneticist
- Specialist screening practitioner (SSP)
- Clinician with expertise in colonic stenting

MDT data in the UK is intrinsically linked to data provided for the National Bowel Cancer Audit. It is mandatory for all patients to be included in both processes and the Audit therefore works to ensure all patients are discussed at MDT[69]. Unfortunately it is not possible from the data to determine what proportion of patients are left out of the MDT/NBCA data but it is likely to be a low percentage.

Patients with rectal cancer tend to be discussed at two or three distinct time points. Firstly, at the time of diagnosis, to discuss histopathology information from biopsies, review staging investigations and consider patient variables as outlined above. At this point a team decision would be made about the requirement for neoadjuvant therapy and which regime should be used. If the plan is for the patient to have surgery only, then the patient may only be discussed twice. Similarly if they undergo neoadjuvant SCRT with a five-day course followed by immediate surgery, then they would only be discussed twice. However, those that undergo neoadjuvant chemoradiotherapy usually have restaging investigations following this treatment.

This allows the response to therapy to be assessed and determines whether there has been any systemic spread of disease in the interim since previous imaging. Their case is then re-discussed prior to planning surgery if indicated. Following surgery all cases are discussed again to review histopathology results and decide whether adjuvant therapy is required. During the follow-up period should there be any need for further discussion, for example to review imaging or decide on the best management for metastatic disease or local recurrence, then the case would be discussed again. For patients who have a clinical complete response to neoadjuvant therapy and decide against operative intervention, instead following a watch and wait policy, their imaging and clinical evaluation results may be discussed multiple times during the course of their follow-up. Figure 1.4 shows these possible time points of discussion in diagrammatic form.

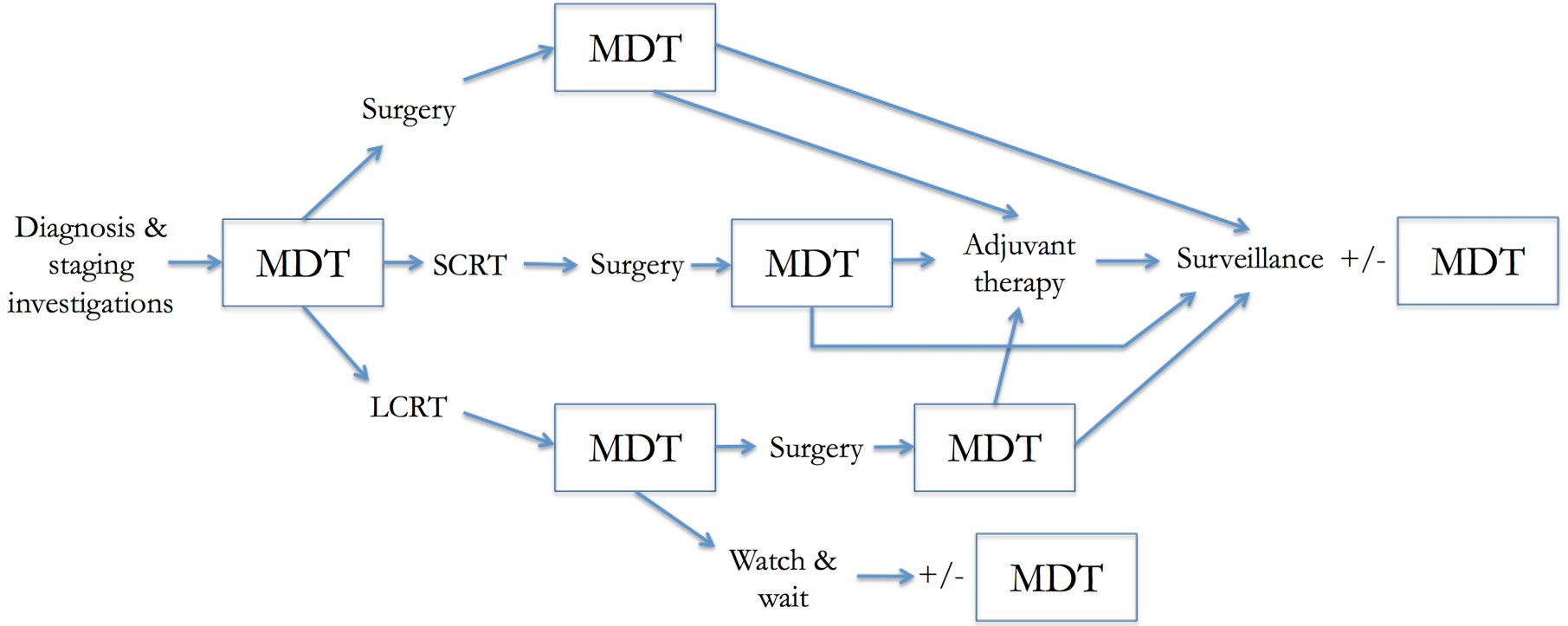
The multiple modalities and treatment approaches available for patients with rectal cancer have made decision making increasingly complex for the healthcare professionals involved in the care of these patients[70]. The improvements seen in survival in recent years have only been possible through an MDT approach to treating rectal cancer[28].

There are both benefits and drawbacks of the MDT process. Despite the widespread implementation of MDT discussions for all cancers, including rectal cancer, there is a lack of evidence for benefit in terms of patient outcomes. The widespread adoption of the system and the corresponding change in mindset of the professionals involved means it would now be impossible to conduct a randomised trial to assess the benefits of MDT discussion[71]. Discussing patients at MDT has been shown to increase the length of time to treatment[72]; it also has a considerable cost in terms of the protected time required from all of the professionals who participate.

Although there have not been any trials conducted, a team in the UK who conducted a retrospective audit, have shown that MDT discussion, based on MRI with implementation of neoadjuvant therapy, reduced CRM positivity rates compared to patients from the preceding two years who had not been discussed at MDT[73]. Another similar audit has shown an increase in the proportion of patients undergoing adjuvant therapy[74]. A further study analysed data from the Swedish Rectal Cancer Registry to show that those patients with stage II or III cancer who were discussed at MDT were significantly more likely to undergo neoadjuvant radiotherapy even when results were corrected for comorbidity and age[75]. Other suggested benefits of the MDT system include[76,77]:

- Patients who are discussed at MDT are more likely to be involved in a clinical trial
- Improved communication and collaboration between the professionals involved
- The MDT provides an educational opportunity for trainees and for the professionals involved
- It provides support from colleagues
- Improves consistency of management across and between teams with the aim of improving outcomes

Figure 1.4 Diagram illustrating time points at which patients undergoing surgery for rectal cancer may be discussed at MDT meeting.
MDT: multi-disciplinary team; SCRT: Short course radiotherapy; LCRT: Long course chemoradiotherapy.



1.6 Surgical approaches to sphincter preservation

Rectal cancer surgery is technically challenging for a number of reasons. The position of the rectum, low down within the pelvis, leads to inherent problems with difficult access. The necessary plane of dissection runs very close to autonomic nerves. Poor visualisation and inadequate control of the distal segment at the time of anastomosis can be a particular problem. Partly because of these reasons, local recurrence rates following rectal cancer surgery are much higher than those for colon cancer. Also because of these problems, the rate of surgical innovation, with the development of new equipment and new approaches is also increased in rectal cancer.

1.6.1 History of sphincter-preserving surgery

Traditionally, excision of the rectum and anus *en bloc*, via an APER was the historical gold standard operation for rectal cancer as described by Ernest Miles in 1908[78]. His proposals were based on a combination of anatomical and biological principles, with a surgical technique based on an understanding of the lymphatic spread of cancer[2]. In 1910, the American surgeon Donald Balfour described the first 'anterior resection' with anastomosis between the colon and rectum[79]. This technique initially showed high mortality rates due to frequent anastomotic leaks[80]. In 1948, Claude Dixon of the Mayo Clinic established the safety of sphincter preservation via anterior resection by publishing his series showing a mortality rate of 2.6% and overall 5-year survival of 64%[81]. Despite this, APER remained the most frequently carried out operation for rectal cancer. Sir Alan Parks established a further development in 1972 with his description of a pull-through technique and hand-sewn coloanal anastomosis, facilitating sphincter preservation even for low rectal cancers[82]. Surgical techniques evolved further with the development of circular stapling equipment in the 1970s[80]. These staplers were designed to get low down into the pelvis to facilitate a low anastomosis.

From the first descriptions of anterior resection until the 1970s, the blunt pelvic dissection used resulted in high local recurrence rates of 30-40%; this in turn led to poor overall survival[83]. In 1982, Professor Bill Heald published the first description of rectal resection with a total mesorectal excision (TME)[84]. The description of this approach led to a marked change in rectal cancer surgery outcomes and has been universally adopted as the gold standard method for resection. A TME approach utilises sharp dissection through embryological avascular plane between the visceral

and parietal pelvic fascia, using anatomical principles, with resection of the mesorectum and the lymph nodes contained within it[84]. The rectum is then removed *en bloc* alongside the blood supply and lymphovascular drainage[84]. Dissection outside of this plane may lead to autonomic nerve injury with resulting bladder and sexual dysfunction resulting from damage to the superior hypogastric plexus & bilateral hypogastric nerves, which join the sacral parasympathetic complex[85].

TME is used for the mid and lower rectum, for tumours in the upper rectum it is acceptable to resect 5cm of mesorectum distally[84]. For upper rectal tumours a TME would create a lower than necessary anastomosis with a subsequent negative effect on functional outcome. TME dramatically reduced the rates of local recurrence to less than 10%[86]. As a consequence of this, 5-year survival also improved, for example Heald *et al.* were able to achieve 80% 5-year survival[86]. TME has also been shown to reduce post-operative bladder and sexual dysfunction[87].

Until the 1980s it was believed that a 5cm distal margin was required when carrying out a resection for rectal cancer. This was initially based on Miles' belief that rectal cancer spreads distally via the lymphatic supply[2]. In the early 1980s it was established that Miles had overestimated the distal spread of rectal cancer and that a distal margin of 2cm was sufficient[88,89]. Williams *et al.* demonstrated that distal spread >1cm occurred in a low proportion of rectal cancers and was associated with poor outcome despite surgery[88]. Subsequent studies demonstrated that for distal tumours an even smaller distal margin of 1cm was sufficient[90] and more recent results have suggested than even this margin may be greater than required[91,92]. In 1986, the importance of circumferential resection margin (CRM) positivity in predicting local recurrence and poor survival was first described[93]. This has now replaced distal margin as the more important predictor of outcome in rectal cancer surgery. In a meta-analysis of clinico-pathological predictors of local recurrence, CRM was shown to be the strongest predictive factor[94]. These developments have all facilitated increased rates of sphincter preservation.

There has been debate in recent years about whether oncological outcomes following APER are inherently worse than those following anterior resection[95]. This resulted from high rates of CRM involvement and local recurrence in some published series[96,97]. A number of explanations have been proposed to explain these findings. Tumours that require an APER were more likely to be distal in the rectum, locally advanced, poorly differentiated, and have poor response to neoadjuvant therapy[98].

Other commentators have suggested that surgical technique may be responsible; as the mesorectum narrows at the level of the pelvic floor, it is possible that inadequate circumferential clearance occurs with so-called 'waisting' of the specimen[95]. With meticulous technique for APER, avoiding CRM positivity and tumour perforation, equivalent oncological outcomes to those for anterior resection have been demonstrated by some studies, with local recurrence rates around 5%[99-101]. There have also been recent moves to amend technique for APER back to the original described principles, with increasing use of extralevator APE (ELAPE)[102].

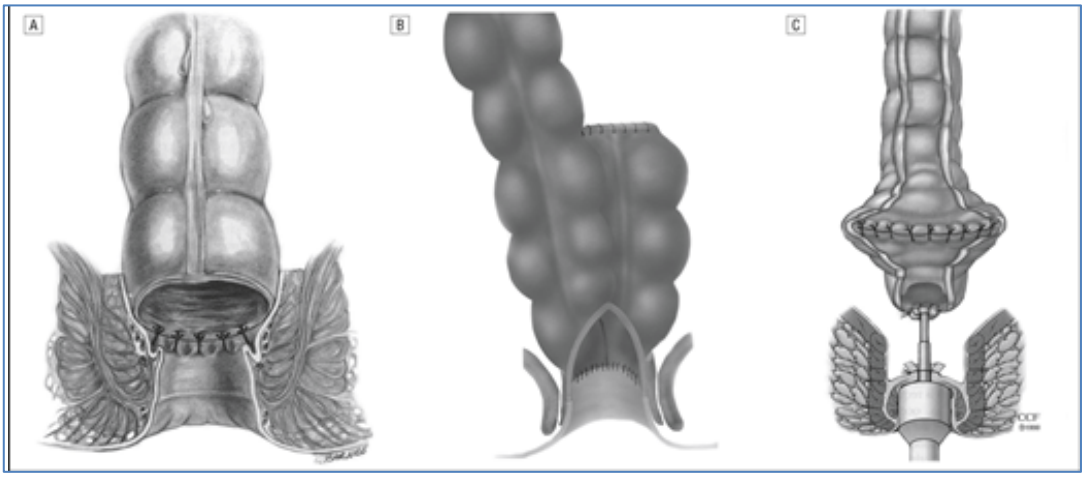
A number of options are available for configuration of anastomoses following an anterior resection (Figure 1.5). These have been investigated predominantly to try and reduce the impact of rectal resection on post-operative function. This is discussed later in the chapter. The most important criteria for any anastomosis are a lack of tension and ensuring adequate vascular supply to the created join.

Morbidity is frequently experienced following TME, with major trials reporting up to 45% of patients undergoing complications[103]. Anastomotic leak following anterior resection remains one of the most serious causes of morbidity and mortality[104], leading to post-operative death from sepsis, reoperation, stoma, prolonged hospital stay and poor quality of life[104]. A further negative outcome from anastomotic leak is that it delays the use of adjuvant therapy. Reported rates vary widely, partly due to inconsistent definition but large prospective databases show the overall rate to be around 10%[105,106]. The main risk factor for anastomotic leak is height of the anastomosis from the anal verge, with anastomoses <7cm at highest risk[107]. Other risk factors include neoadjuvant therapy, male gender and smoking[106].

A defunctioning stoma, which may be a transverse colostomy but is more usually a loop ileostomy, is often created to protect against the negative clinical sequelae of an anastomotic leak. A number of meta-analyses of studies randomising patients to undergo defunctioning stoma have shown that a temporary stoma reduces the clinical consequences of anastomotic leakage and reduces the risk of reoperation, although it does not appear to reduce mortality[109-112]. Whether the benefit of a defunctioning stoma remains in an era of enhanced recovery from surgery has been questioned[113]. Rates of defunctioning are highly variable between countries and centres. As discussed above, audit data and retrospective studies have demonstrated that up to 25% of temporary stomas are not reversed[7]. Delay of closure beyond 6 months has been shown to increase the complications resulting from closure[114] but

the effect that delayed closure may have on post-operative bowel function is unknown.

Figure 1.5 Options for anastomotic configuration following anterior resection: A) Straight coloanal anastomosis; B) Colopouch-rectal anastomosis and C) Coloplasty-anal anastomosis[108].



Concern about anastomotic complications and poor post-operative function, may lead surgeons to carry out a Hartmann's procedure for rectal cancer. Although these stomas are labelled as temporary, many of them are created with no intention to reverse them. There is considerable morbidity related to reversal of a Hartmann's procedure and less than 10% of Hartmann's procedures carried out for cancer in England are reversed[6].

1.6.2 Laparoscopic surgery

Over the last 20 years, laparoscopic resection has been widely adopted for the resection of colorectal tumours. A number of randomised trials have examined the role of laparoscopic surgery in the resection of rectal cancer and have shown conflicting results regarding the effect on pathological and oncological outcomes. The UK Medical Research Council trial was one of the first studies of conventional versus laparoscopic assisted surgery in colorectal cancer[115]. It showed a conversion rate of 34%, comparable morbidity and mortality, and no difference in recurrence rates or survival[115]. In the initial results there were concerns raised over the rates of CRM positivity in the laparoscopic anterior resection group, 12% vs. 6% in the open group[115]. However long-term results continue to show equivalent oncological outcomes in terms of comparable overall and disease free survival[116].

The more recently reported European COLOR II randomised trial of laparoscopic vs. open surgery for rectal cancer included 1044 patients and showed similar results, with comparable morbidity, mortality, recurrence rates and 3-year survival[117]; the conversion rate was lower at 16%. Following this, a Cochrane review of laparoscopic versus open total mesorectal excision for rectal cancer concluded that there was moderate evidence that laparoscopic and open surgery have similar long term survival outcomes, and that laparoscopic surgery may have benefits for short-term recovery[118].

The ALaCaRT randomised clinical trial studied the effect of laparoscopic-assisted resection versus open resection on pathological outcomes in rectal cancer[119]. This multicentre study was carried out in Australia and New Zealand and included 475 patients. A composite end point of adequate surgical resection was used and this was defined as (1) complete total mesorectal excision, (2) a clear circumferential margin (<1 mm), and (3) a clear distal resection margin (<1 mm). The study failed to establish the non-inferiority of laparoscopic resection. The CRM negative rate was 93% in the laparoscopic group vs. 97% in the open group and complete TME rate was 87% in the laparoscopic group vs. 92% in the open group[119]. The conversion rate from laparoscopic to open surgery was 9%. Recently published results show no difference in 2-year local recurrence, overall survival or disease free survival between the open and laparoscopic groups[120].

The ACOSOG Z6051 trial was a study of 486 patients from the USA and Canada with a similar design to ALaCaRT, looking again at pathological outcomes in rectal cancer following open or laparoscopic resection[121]. It used a similar composite end-point including the same three criteria as ALaCaRT and also failed to establish non-inferiority of the laparoscopic approach[121]. Successful resection occurred in 81.7% of laparoscopic cases and 86.9% of open cases[121]. The 2-year local recurrence and disease free survival rates for the Z6051 trial have also been recently published, and similarly show no difference between the laparoscopic and open resection groups[122].

Authors from both the ALaCaRT and Z6051 studies advised that the results do not support routine use of laparoscopic resection for all patients[119,121], although neither study was powered to demonstrate the superiority of either an open or laparoscopic approach, and both show similar mid-term oncological outcomes for laparoscopic and open surgery[120,122]. The validity of the composite endpoint used in these studies

has also been questioned, and suggested as a possible reason for the conflicting results when compared with the COLOR II trial[123].

As is the practice during many of their surgical approaches, surgeons in Japan practise extended lymphadenectomy during TME surgery, carrying out *en bloc* excision of lateral pelvic lymph nodes as their standard approach[124]. The incidence of metastasis in these lateral pelvic nodes has been shown to be around 15%[125] and has been linked to increased rates of local recurrence[126]. Retrospective studies have not shown any survival benefit from routinely carrying out lateral nodal dissection[125], although comparisons with Western surgical results and recurrence rates are hindered by differing anatomical definitions[127]. A large multicentre randomised trial of TME vs. TME with lateral lymph node dissection showed no significant difference in 5-year recurrence-free survival but significantly higher local recurrence with TME alone[128]. Although it may therefore seem that lateral dissection may provide benefit, the results of this study are not necessarily applicable to the UK population as none of the patients received neoadjuvant therapy[129].

1.6.3 Robotic-assisted laparoscopic surgery

Robotic-assisted laparoscopic surgery was first developed in the 1990s and was initially used by urological surgeons for prostatectomy[130]. The more advanced robotic surgical platforms that are used currently have been designed for multi-quadrant surgery and offer theoretical advantages for pelvic surgery including the use of a 3-dimensional view of the operative field, finer dissection with the freely articulating EndoWrist™ equipment, intuitive instrument handling and precise movements aided by tremor filtration[130]. This improved visualisation and precision in dissection could theoretically facilitate improved preservation of autonomic nerves in the pelvis during TME, and could possibly lead to a higher rate of sphincter preservation and improved functional outcomes[130]. Studies assessing functional outcomes following robotic surgery have so far used non-randomised approaches and only have a very small number of patients included[130]. The largest randomised study of robotic surgery for rectal cancer, the ROLARR trial, did not show any short-term benefit for robotic-assisted surgery[131]. However, the primary end point of the ROLARR study was conversion rate to open surgery and longer-term oncological and functional outcomes are not yet reported.

1.6.4 Sphincter preserving techniques

Recent developments mean that a range of surgical options is now available for sphincter preservation.

In selected cases of rectal cancer, local excision may be considered as an alternative to radical resection. There are several different techniques used for local excision, of which the most frequently studied is transanal endoscopic microsurgery (TEM)[132]. This technique was initially pioneered by Gerhard Buess who first described the technique in 1983[133] and was further investigated in patients who were considered unfit to withstand the morbidity and mortality associated with a major resection[132]. Local excision has subsequently been investigated in early tumours and the most difficult aspect of this surgery remains selecting appropriate patients[132,134]. The principle of local excision involves excision of the rectal tumour with a full-thickness excision of the rectal wall but no removal of local nodes[28]. Recurrence rates were initially very high and have remained the major concern about the adoption of this technique[135]. The majority of T1 and T2 tumours will be node negative but the assessment of nodal involvement is not completely accurate, as has been covered above. A subset of the patients who undergo TEM will, therefore, have positive nodes left behind, leading to the increased rates of local recurrence seen in these patients[136].

A meta-analysis of outcomes following TEM in patients with T1 tumours showed that TEM was safer than undergoing a major resection, with 0% mortality, lower morbidity and shorter hospital stay[137]. Local recurrence following TEM was significantly higher than that following radical resection but there was no difference in survival at five years[137]. For T2N0 tumours, the use of local excision is more controversial. A recent study showed local recurrence rates of 29% in local R0 excision of low-risk T2 tumours[138]. A pooled analysis has shown increasing risk of recurrence with increasing T stage, with recurrence rates of 0% for T0, 2% for T1 and between 6 and 20% for T2 tumours[139]. Risk factors for recurrence have been analysed and include depth of invasion, tumour diameter, lymphovascular invasion and poor differentiation[140].

A further recent meta-analysis has shown, however, that in patients undergoing neoadjuvant chemoradiotherapy, the local recurrence rates, disease free and overall survival were equivalent between those undergoing CRT and local excision and

those having CRT followed by radical resection[141]. The most frequent complications following TEMs are suture line dehiscence, bleeding and urinary retention[136]. Inadvertent entry into the peritoneal cavity occurs in 5.8% of cases[142]. Patients undergoing neoadjuvant CRT followed by local excision are more likely to develop wound related complications including dehiscence[143].

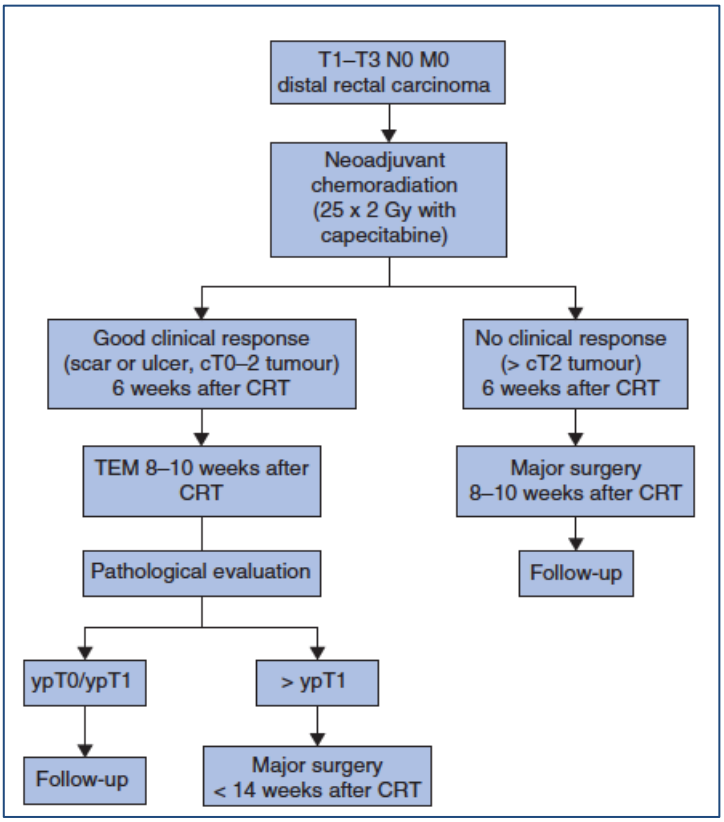
A number of clinical trials have investigated the role of local excision. The American College of Surgeons Oncology Group (ACOSOG) Z6041 study was a single-arm study to assess the safety of local excision for T2N0M0 tumours[144]. There was a high rate of complete pathological response (44%) and high rates of negative resection margins[144]. Also, the rate of complications during CRT and following local excision was higher than anticipated at 39%; long-term oncological outcomes are awaited[144]. The recently published Chemoradiation therapy for rectal cancer in the distal rectum followed by organ-sparing transanal endoscopic microsurgery (CARTS) study was a non-randomised study including patients with T1-T3/N0 tumours[145]. A flow chart from this complex study is shown in Figure 1.6. The results of the study showed that, in half of the patients with rectal cancer, following this protocol enabled them to achieve organ preservation. Toxicity to therapies was again a major issue: 42% of patients experienced toxicity to chemoradiotherapy and 28% had complications from surgery post TEM[145].

The GRECCAR 2 study randomised T2-T3/N0 patients with a good response to neoadjuvant chemoradiotherapy to local excision vs. TME[146]. The results of the study showed no significant difference in survival, local recurrence or morbidity between the two groups. However, 35% of the local excision group underwent a completion anterior resection and the investigators acknowledged that this made the results difficult to interpret[146].

Ongoing studies, the results of which will further inform the debate surrounding the use of local excision, include:

- TEM and Radiotherapy in Early Rectal Cancer (TREC)[147]: a randomised trial of TME vs. neoadjuvant short course radiotherapy followed by delayed local excision at 8 to 10 weeks, for T1/T2N0 tumours.
- The study groups from TREC and CARTS have now combined their protocols to undertake a trial that randomises patients between 3 arms. The STAR-TREC will randomise to: standard TME; short course radiotherapy with TEM; and long course chemoradiotherapy with TEM[142].

Figure 1.6 Protocol from the CARTS study (Chemoradiation therapy for rectal cancer in the distal rectum followed by organ-sparing transanal endoscopic microsurgery)[145]. CRT: chemoradiotherapy; TEM: transanal endoscopic microsurgery.



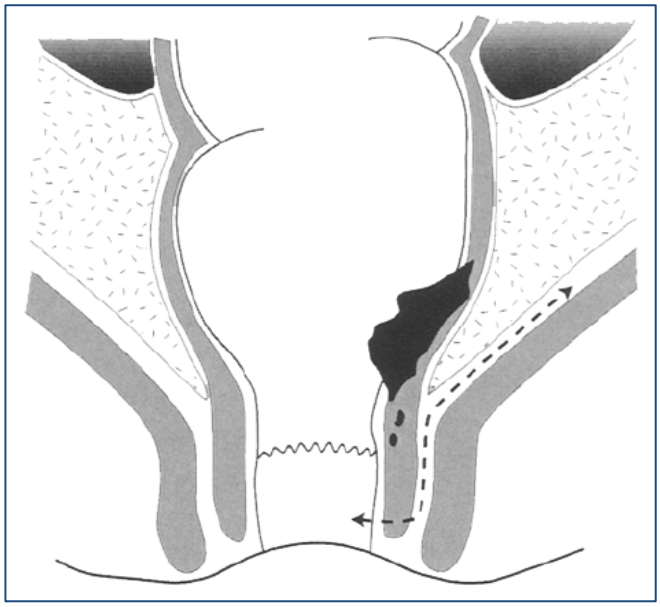
The use of the anterior perineal plane to resect the distal rectum was initially described by Cuneo in 1908 and published in a French surgical textbook in 1926[148] as quoted in a paper from France in 1988[149]. The French surgeons went on to utilise the procedure themselves, publishing a large case series[149]. It is unclear why there was no further uptake of the procedure following this report. Use of the anterior perineal approach to perform a rectal anastomosis has been reported intermittently since then[150].

Intersphincteric dissection to facilitate removal of low rectal tumours is a procedure that developed from methods used in surgery for inflammatory bowel disease[151,152]. The procedure involves a transanal approach with dissection into the intersphincteric plane (see Figure 1.7) and either partial or total excision of the internal anal sphincter usually followed by a hand-sewn anastomosis[151]. Rullier *et al.* have used this procedure for type II juxta-anal and type III intra-anal lesions in the classification, which they proposed (shown in Figure 1.3) and have demonstrated

that with use of this procedure, it is possible to avoid a permanent colostomy for the majority of patients with low rectal cancer[153].

Systematic reviews show outcomes comparable to those of other techniques for low rectal cancer with low operative mortality of 0.8 to 1.6%, rates of anastomotic leakage of 10.5%, local recurrence of 6.7 to 9.5% and 5-year survival rates of up to 86%[151,154]. There have been concerns about the effect that this procedure is likely to have on functional outcomes and proponents agree that patients need to have good pre-operative function and be motivated to deal with ensuing continence issues[151]. Meaningful comparisons between the functional results of sphincter-preserving procedures have been hindered by a lack of validated outcome measures[155]. Bretagnol *et al.* compared functional outcomes with those following a hand-sewn coloanal anastomosis (which would be the only feasible sphincter-preserving alternative for many of the patients undergoing this surgery)[156]. The results showed higher rates of faecal incontinence following intersphincteric resection with some effect on quality of life; there was no difference in other measures including stool frequency and urgency[156].

Figure 1.7 Plane of dissection (indicated by dotted line) for intersphincteric resection with subtotal excision of the internal anal sphincter[157].



Transanal TME (sometimes known as transanal minimally invasive surgery – TAMIS) evolved from a combination of technologies including transanal endoscopic microsurgery (TEM); endoscopic mucosal resection (EMR); single incision laparoscopic surgery (SILS) and natural orifice transluminal endoscopic surgery

(NOTES)[158]. This approach to resection can be used with a transanal approach only or as a combined approach with initial transanal dissection followed by a standard abdominal approach which is then extended distally to join up the resection[159]. The transanal approach is used predominantly for tumours within 5cm of the dentate line and starts with the definition of a tumour-free distal margin[159]. It is believed to best suit patients in whom an abdominal approach alone would be very difficult and would limit the ability to carry out a sphincter-preserving resection; these include male patients, obese patients and those with a narrow pelvis[159]. With the development of equipment designed for this procedure, early problems with port dislodgement, maintenance of a pneumoperitoneum and achieving adequate exposure are gradually being resolved.

The first report from the International transanal TME registry reported on 720 cases and shows results equivalent to those for standard TME in terms of operative time, complete resection and good macroscopic quality of the TME specimen as well as short-term morbidity and mortality[160]. 32% of cases in this series used two teams operating simultaneously from transanal and abdominal approaches; the abdominal part of the TME was conducted laparoscopically for 82% of cases[160]. Long-term oncological and functional results following this procedure are not yet reported; the International registry aims to report initial findings at three years. There is also an ongoing randomised trial of laparoscopic vs. transanal TME for mid/low rectal cancer, the COLOR III trial, which should provide further data on outcomes[161].

Proponents of perineal approaches believe that these techniques will be widely adopted and are the next major development to advance the practice of rectal cancer surgery[162]. A randomised trial of abdominal vs. perineal approach for distal rectal dissection in 100 patients with low rectal cancer was carried out with a primary endpoint of surgical quality, and showed significantly lower rates of CRM positivity in the perineal group (4 vs. 18%), with no difference in morbidity[163]. Critics feel the studies that have been carried out so far have been done on patients with early favourable tumours, that many complications are unreported, and that case selection bias is an issue in the majority of publications of this method. There is undoubtedly a steep learning curve, during which care must be taken to avoid complications[164]. It is likely that the rectal cancer surgeon needs to be aware of all these available approaches and techniques which could be used in difficult cases to help minimise the poor outcomes associated with an involved CRM.

1.7 Tumour biology, patient factors and molecular biomarkers

1.7.1 Tumour biology

Over 95% of tumours that arise in the colon and rectum are adenocarcinomas[165]. A proportion of carcinomas are sub-classified as either mucinous adenocarcinoma, where over 50% of the tumour volume is composed of extracellular mucin or signet-ring cell carcinomas, defined by the presence of > 50% of tumour cells with prominent intracytoplasmic mucin[166]. Early histological analysis allows confirmation of the diagnosis of adenocarcinoma and rules out other rarer tumour types, which include squamous cell carcinomas, adenosquamous carcinomas, neuroendocrine carcinomas, sarcomas, lymphomas, carcinoid tumours, melanomas and gastrointestinal stromal tumours[165,166].

CRC arises via a sequence from adenoma through to carcinoma due to genetic and epigenetic alterations in cell growth, differentiation, proliferation and apoptosis[167]. This process is partly controlled by the action of oncogenes and tumour suppressor genes[167]. Mutations in the adenomatous polyposis coli (*APC*) gene are an early step and have been demonstrated in over 70% of sporadic CRCs[168].

Sporadic CRC progresses by two different pathways of genomic destabilisation: chromosomal instability (CIN) and microsatellite instability (MSI)[169]. MSI results from defects in the DNA mismatch genes leading to the accumulation of insertion and deletion errors in microsatellite repeat sequences[170]. This abnormality is found in about 15% of sporadic CRCs and a much higher proportion of hereditary cases[170]. These tumours are more often located in the proximal colon, are more often mucinous, are less frequently associated with metastases and have a better prognosis[171-174]. CIN cancers make up around 45% of CRCs and are defined by chromosomal abnormalities[175]. The remaining 30-40% of sporadic cancers have been classified as microsatellite and chromosome stable (MACS)[176].

Genetic susceptibility to CRC due to hereditary factors is believed to account for around 5 to 15% of cases[177]. These hereditary factors, affecting relatives of those with CRC are mainly composed of polymorphisms with combinations of gene variations resulting in increased risk[178]. Mendelian familial syndromes involving an identified single gene defect make up around 2 to 6% of CRC cases and therefore affect a minority of patients[179]. These syndromes are usually divided into polyposis and non-polyposis syndromes. Polyposis syndromes include familial adenomatous polyposis

(FAP) with mutation of the *APC* gene; MYH-associated polyposis (MAP) with mutation of the *MYH* gene; and Peutz–Jeghers syndrome with mutations of the *STK11* gene[179]. Hereditary non-polyposis colon cancer (HNPCC), now called Lynch Syndrome (LS) is an autosomal dominant syndrome resulting in early CRCs. LS is caused by defective DNA mismatch repair genes, *MLH1, MSH2, PMS2* and *MSH6*, and CRCs arising as a result of HNPCC are characterised by MSI[179].

Chronic inflammatory bowel problems, ulcerative colitis (UC) and Crohn's disease also increase the risk of CRC[180]. Longstanding inflammatory changes lead to dysplasia, which in turn leads to carcinogenesis[180]. For UC, the risk of CRC is 2.4x that of the normal population, with male patients and those diagnosed at a young age, as well as individuals with extensive colitis, having the highest risk[181].

1.7.2 Pathological factors affecting outcome

Several histopathological tumour characteristics have been shown to have an important bearing on outcome in rectal cancer. Grading of rectal cancer is based on the predominant degree of differentiation in the primary tumour and has been shown to affect recurrence rates and survival[182,183]. A number of studies have investigated the possible effect of tumour location circumferentially within the rectum[184,185], these have shown conflicting results and this lack of effect has been confirmed by further research[186]. However, tumour height within the rectum has consistently been shown to affect outcome with increased recurrence rates and poorer survival for lower rectal tumours[187,188].

Lymph node involvement has an impact on recurrence and survival in rectal cancer, therefore justifying its inclusion in the staging classification[187,188]. However, the number of lymph nodes resected in patients undergoing surgery also has prognostic significance[189], mainly because this number acts as a surrogate marker of surgical and pathological excellence[189]. The ACPGBI guidelines for CRC indicate that resection specimens should include at least 12 nodes[38] although it is widely accepted that neoadjuvant therapy reduces the lymph node yield[190]. According to the principles of TME surgery, if the nodes are taken along with the rectum *en bloc* then involvement of lymph nodes should not have such prognostic significance and other factors, particularly involvement of the CRM, should have greater significance[191]. For these reasons guidelines advise that the quality of the TME specimen should be included in pathology reporting and this data is included in reporting proformas[38]. The CR07

trial of pre-operative short course radiotherapy vs. post-operative long course chemo-radiotherapy showed that the quality of the TME specimen has a bearing on recurrence. In patients with an involved CRM and a poor quality specimen, recurrence was 12%, falling to 4% in those with a mesorectal plane of dissection[192].

The presence of extramural venous invasion (EMVI) either on MRI or on pathological assessment has been shown to lead to significantly higher rates of local recurrence and significantly poorer disease free survival[188,193]. Similarly, identification of extramural perineural invasion on pathological review also increases the rate of recurrence and worsens disease free survival[194-196].

Current protocols for locally advanced rectal cancer include neoadjuvant therapy with chemo- and radiotherapy. The evidence for this treatment and the different regimes used will be discussed further below. The response of individual tumours to chemo- and radiotherapy is variable and unpredictable. Some tumours fail to respond at all and in fact progress during neoadjuvant therapy. The optimal response is pathological complete response (pCR), with total regression of the tumour. This occurs in 10-30% of cases and can facilitate sphincter preserving surgery or even organ preservation by avoiding surgery altogether. The ability to predict the response of a tumour to neoadjuvant therapy would allow treatment to be tailored on an individual basis. For patients who would not benefit, they could avoid the time, cost and toxicity of these therapies. For patients with highly radiosensitive and chemosensitive tumours, the neoadjuvant regime could actually be intensified with the aim of avoiding surgical intervention and following a treatment protocol more akin to that for anal carcinoma.

1.7.3 Patient factors affecting outcome

Making decisions about patients undergoing low rectal surgery with respect to sphincter preservation is complex and takes into account several different factors relating to the patient. Some of these such as age, current bowel function, neo-adjuvant treatment and obstetric history relate to their likely functional outcomes. Others, such as smoking status, nutritional state and co-morbidities relate to their likelihood of complications following the surgery. In individual decision-making, the wishes of the individual patient may determine the decision made about the type of surgery.

The gender, body mass index (BMI) and body shape of a patient all affect their pelvic anatomy. The narrow male pelvis makes sphincter-preserving surgery technically challenging; this in combination with a high BMI may lead to reconsideration of the operative approach, for example prompting a change to a transanal technique[197]. Obesity is associated with increased perioperative morbidity[198]. Despite the potential effect it could have on outcome, it has previously been poorly recorded but BMI has now been included in the NBCA dataset (from 2013-14 onwards), which should improve routine recording[5]. In the first round of the data collection, this variable was recorded in 36.5% of those undergoing major surgery[199].

The age of a patient is always a consideration but physiological age is much more important to fitness to undergo major surgery than numerical age. In recognition of this, the routine assessment of performance status and cardiovascular fitness has become normal practice. Cardio-pulmonary exercise testing (CPEX) was traditionally used prior to oesophago-gastric and hepatobiliary and pancreatic surgery. At some NHS Trusts it is used for selected high-risk patients who are considered to need assessment prior to colorectal resection. This decision is sometimes made by the surgical team and sometimes advised by the MDT. As for BMI, CPEX status has recently been included as a variable within the NBCA dataset, unlike BMI however, this data was poorly recorded with only 2.5% of those undergoing major surgery being included in first round of data collection[199]. It is possible that this reflects a low percentage of patients undergoing CPEX or that this data is not available to the MDT for recording within the audit data. Performance status, which has been routinely used by oncologists for many years as a measure of fitness for adjunctive therapy, was much better recorded, at over 80%[5]. These variables have been included in an effort to better understand the potential reasons why some patients do not undergo surgery[199].

A patient's comorbidities are also important to their ability to undergo treatments for rectal cancer including chemotherapy, radiotherapy and surgery. The effect of medications needs to be considered and management of any coexisting conditions may need to be optimised prior to surgery. Information relating to their social circumstances should also be recorded and can be useful in an MDT setting when discussing what support the patient may need as they progress through treatment.

Baseline anorectal function should also be included within the history for all patients with rectal cancer. This needs to be differentiated from any effect the rectal tumour

has had on function[200]. Details may include any pre-existing symptoms such as incontinence, urgency, or rectal evacuatory dysfunction; prior obstetric injury; anorectal surgery or neuromuscular problems. If there are any concerns over pre-treatment sphincter function, then formal assessment may be carried out with anorectal manometry, rectal sensory testing, endo-anal ultrasound and proctography.

1.7.4 Assessing response to therapy

The response of a tumour to neoadjuvant therapy can be assessed in different ways. Tissue regression grading (TRG) systems stratify response based on the effects of radiotherapy, with the grade determined on pathological analysis by the ratio of fibrosis to tumour[201]. Many different TRG classifications have been proposed; they have all been shown to have association with outcome but there is no consensus about which system should be used[202]. Mandard initially proposed the classification for oesophageal cancer; this scale and variations on it remain the most widely used TRG scoring system[202]. The Mandard score goes from TRG 1 indicating complete response through to TRG 5 indicating no regression[203]. The Mandard score was subsequently modified by Dworak *et al*. for use in assessing rectal cancer response[204] and has subsequently been modified and adapted several times[202]. The descriptions within the scoring systems overlap considerably but an extra layer of confusion is added because in some systems low numbers reflect good response and others run the opposite way with high TRG grades reflecting the best response (see Table 1.3). A recent study comparing the accuracy of the different systems found the four-tier system approved by the AJCC to most accurately predict recurrence[202]. The ACPGBI Guidelines on CRC provide a dataset for pathology reporting which endorses and recommends the system described in the Royal College of Pathologists Guidelines (see Table 1.3)[38].

Downstaging in TNM stage following neoadjuvant therapy is also used as a marker of tumour response. This is hindered by the limitations in the imaging modalities themselves (see above discussion), particularly with regard to nodal assessment. Despite this, downstaging has been shown to significantly predict good outcomes[205] and is frequently used in the assessment of response.

Table 1.3 Comparison between different tissue regression grading systems [202] AJCC: American Joint Committee on Cancer; MSKCC: Memorial Sloan-Kettering Cancer Center; TRG: tissue regression grade.

Tier	Mandard[203]	AJCC [36]	Dworak[204]	Rödel[206]	MSKCC[207]	American College of Pathologists[208]	The Royal College of Pathologists[209]
TRG 0		No residual tumour cells	No regression	No regression		No viable cancer cells	
TRG 1	No residual cancer	Single cell or small groups of cells	Predominantly tumor with significant fibrosis and/or vasculopathy	Minor regression (dominant tumor mass with obvious fibrosis in 25% or less of the tumor mass)	100% Tumor response	Single cells or small groups of cancer cells	No viable tumour cells (fibrosis or mucus lakes only)
TRG 2	Rare residual cancer cells	Residual cancer with desmoplastic response	Predominantly fibrosis with scattered tumor cells	Moderate regression (dominant tumor mass with obvious fibrosis in 26% to 50% of the tumor mass)	86%-99% Tumor response	Residual cancer outgrown by fibrosis	Single cells or scattered small groups of cancer cells
TRG 3	Fibrosis outgrowing residual cancer	Minimal evidence of tumor response	Only scattered tumor cells in the space of fibrosis with/without acellular mucin	Good regression (dominant fibrosis outgrowing the tumor mass; i.e., more than 50% tumor regression)	≤85% Tumor response	Minimal or no tumor kill; extensive residual cancer	Residual cancer outgrown by fibrosis
TRG 4	Residual cancer outgrowing fibrosis		No viable tumor cells detected	Total regression (no viable tumor cells, only fibrotic mass)			Minimal or no regression (extensive residual tumour)
TRG 5	Absence of regressive changes						

1.7.5 Molecular biomarkers

To identify potential predictive biomarkers of response to neoadjuvant therapy, work has concentrated on histological and molecular assessment of pre-treatment biopsies[201]. There are currently no reliable biomarkers available to stratify patients although many markers have been investigated and the findings from these studies are discussed below. Response to therapy is complex, involving multiple pathways and, as such, it is unlikely that any one marker can entirely predict response in all cases[210]. There are also only a limited number of studies that look at panels of markers with cohorts of sufficient numbers. Investigations are often retrospective, underpowered with far too few patients, and do not include both technical and independent validation[211]. Further problems with reproducing the results of previous studies arise from a lack of consistency between treatment regimes and because the assessment of response is carried out using a variety of systems (see above).

One of the main factors accounting for the differing sensitivity of individual tumours to radiotherapy is tumour hypoxia. Hypoxia is a hallmark of solid tumours and plays an important role in tumour growth and progression. It arises due to angiogenesis being insufficient to supply the needs of the rapidly proliferating tumour cells and their increased consumption of oxygen[212]. As the tumour outgrows the local blood supply, adaptation towards hypoxia allows it to continue to grow. Cellular adaptations to hypoxia are mediated by genes activated by transcription factors known as hypoxia-inducible factors (HIFs)[213]. Hypoxic tumours are more resistant to radiotherapy since oxygen free radicals are required for the generation of DNA damage, such as DNA double strand breaks, caused by ionizing radiation[213]. Hypoxic tumours are also resistant to chemotherapy via a number of mechanisms. Some chemotherapeutic agents depend on cellular oxygenation for their mechanism of action; others act on tumour cells during DNA synthesis and are made less effective by the slow cell cycling occurring as a result of hypoxia[212]. Because of these links between hypoxia and response to CRT, markers of hypoxia may be useful as biomarkers of response to CRT.

A further line of investigation into the mechanisms behind resistance to therapy is the behaviour of cancer stem cells. These cells within a tumour are able to self renew and differentiate into different cancer cell types accounting for the heterogeneity seen within tumours[214] If the cancer stem cells are not all killed by radiotherapy then

repopulation between treatments or at the end of therapy may reduce overall sensitivity of the tumour to radiotherapy[215].

p53 is a tumour suppressor gene, frequently mutated in CRC (~50% of cancers), which has been widely investigated with possible links to response[201]. Often referred to as the guardian of the genome, it acts by mediating cell cycle arrest and apoptosis in damaged or mutated cells[216]. Direct sequencing has been used to show that mutant p53 genotype is significantly associated with radioresistance[217] and is more frequently found in non-responders[218]. A recent meta-analysis identified 30 studies investigating the relationship between p53 status and response to neoadjuvant therapy in rectal cancer[219]. The overall conclusion was that wild-type p53 status is predictive of good response and increased rates of complete response[219].

The p21 protein has a key functional role in the p53 signalling pathway[201]. Its expression is mainly controlled by p53 and p21 is upregulated by p53 activation secondary to DNA damage (as occurs during radiotherapy), leading in turn to cell cycle arrest[220]. As a result, p21 has been investigated as a marker of radioresistance. p21 can act as both a tumour suppressor and oncogene; its induction and effects on the cell cycle can be mediated via p53-dependent and p53-independent mechanisms[221]. Increased p21 expression has been shown to link to poor response to neoadjuvant therapy[222,223] and worse disease free survival[224,225]. Some studies show no association between p21 expression and outcomes and there has not yet been any attempt at meta-analyses of the studies[226]. Others suggest a more complicated relationship between p53 and p21 expression and the situation is likely to be complex[227].

Epidermal Growth Factor Receptor (EGFR) is expressed in cancers and is linked to aggressive tumours, radioresistance and poor prognosis[228]. It is involved in many cellular pathways including differentiation, proliferation and apoptosis[229]. Giralt *et al.* have shown that EGFR expression is less likely in patients with pCR, more likely in those with poor response and predicts poor disease-free survival[230]. A further study of 183 patients with rectal cancer undergoing neoadjuvant therapy demonstrated on logistic regression that the significant predictive factor of tumour downstaging was a low level of EGFR expression[231].

Cyclooxygenase-2 (COX-2) mediates tumour invasiveness and metastasis[232]. It is an enzyme mediating conversion of arachidonic acid to prostaglandins, which tumour

cells use to protect against radiotherapy induced cell death[211]. Patients with overexpression are more likely to demonstrate poor response[232]. Further studies have shown that COX-2 overexpression is associated with poor TRG[233], increased risk of nodal positivity and significantly shorter survival time[234]. Clinical studies have investigated the potential of drugs that inhibit COX-2 including aspirin and non-steroidal anti-inflammatories. Selective COX-2 inhibitors, such as celecoxib and etoricoxib, have additionally been investigated for the chemoprevention of CRC[235].

A number of other markers have also been investigated. Bcl-2 and bax are proteins involved in cell apoptosis and survival[201]. Bcl-2 is linked to cell survival and expression has been shown to link to increased rates of complete response[201]. Loss of bax function has been linked to chemoresistance and higher levels of expression have been linked to improved response[236]. Ki67 is a marker for proliferation[237]; Jakob *et al.* have shown that rectal cancers with low expression have better response to 5-fluorouracil (5-FU) based therapy[238]. This marker has also been looked at extensively in relation to response to neoadjuvant therapy in other cancers, particularly breast cancer[239].

Thymidylate synthase is involved in DNA synthesis and is the main intracellular target of the chemotherapy drug 5-FU and its derivatives[211]. This explains the finding that overexpression is linked to 5-FU resistance[240,241]. Other markers investigated include circulating tumour cells[210], Cyclin D1[211], Survivin and dihydropyrimidine dehydrogenase[211].

Multiple studies have looked at the association between pre-treatment CEA levels in serum and response to neoadjuvant therapy; these are summarised in Table 1.4 below. A number of studies have also shown that a reduction in CEA levels following neoadjuvant therapy is also associated with a better response to therapy[242-245]. Raised preoperative CEA has also been shown to be a predictor of decreased overall survival[246].

Several studies have attempted to identify a gene expression profile associated with response to neoadjuvant therapy. Ghadimi *et al.* identified a panel of 54 genes, which were able to predict response in terms of tumour downstaging[253]. Rimkus *et al.* have conducted a similar study with similar results, however there was no crossover in the genes identified by these different studies[254]. A further study identified an expression profile, which showed 84% accuracy for predicting pCR, and even greater

Table 1.4 Summary of studies into CEA as a predictor of response to neoadjuvant therapy. CEA: Carcinoembryonic antigen; ng/ml: nanogram per millilitre; pCR: pathological complete response. No.: number of participants; TRG: tissue regression grade; 5-FU: 5-fluorouracil.

Author	No.	CEA level	Treatment	Endpoint	Comment
Park[247]	141	5 ng/ml	45 Gray and 5-FU or doxifluridine	Nodal status TRG	CEA >5 ng/ml associated with increased nodal infiltration and poor response
Das[248]	562	2.5 ng/ml	45 Gray and 5-FU or capecitabine	Downstaging pCR rates	CEA > 2.5 ng/ml was associated with reduced pCR and lower downstaging rates
Moureau-Zabotto[249]	168	5 ng/ml	45 Gray and 5-FU	pCR rates Downstaging	CEA <5 ng/ml associated with pCR and increased downstaging
Perez[250]	170	5 ng/ml	50.4 Gray and 5-FU with folinic acid	pCR rates Disease stage	CEA <5 ng/ml associated with earlier stage and increased pCR rates
Wallin[251]	530	5 ng/ml	45 Gray + 5.4 Gray boost and 5-FU	pCR rates	CEA <5 ng/ml associated with increased pCR rates
Restivo[252]	260	5 ng/ml	45 Gray + 9 Gray boost and 5-FU or capecitabine	pCR rates	CEA <5 ng/ml associated with increased pCR rates

accuracy on a separate validation set[255]. These studies show potential but the complexity of the data produced makes replication and clinical application difficult.

MicroRNAs are small non-coding RNAs that function as regulators of gene expression; it is believed that up to 30% of human genes are microRNA targets[256].

Their regulatory function over oncogenes, tumour suppressor genes and other processes including differentiation, invasion and adaptations leading to resistance to CRT, gives them potential as biomarkers and therapeutic targets. In rectal cancer, microRNAs have been shown to have potential as biomarkers of response to neo-adjuvant CRT. Previous studies using expression arrays on tumour samples from responders and non-responders have identified a number of possible microRNA targets, but these show little overlap and have not been externally validated[257-259].

1.8 Neoadjuvant and adjuvant therapies

The use of adjunctive therapy for rectal cancer began with studies in the 1980s into the use of chemotherapy and/or radiotherapy to reduce the risk of local recurrence and prolong survival[260]. The use of neoadjuvant and adjuvant therapy has continually evolved since that time and the choice of therapy for individual patients remains one of the most complex decisions to be made by the MDT. The treatment chosen for each patient remains dependent on staging modalities and is therefore subject to the limitations of these modalities, which has been discussed above. The unpredictable response of each individual tumour to these therapies also continues to be a further issue. Studies of adjunctive therapy have focussed on answering a series of questions:

1) Should we give chemotherapy or radiotherapy or both?
2) Should we give these therapies before or after surgery?
3) If we give radiotherapy should this be short course or long course and what dose is optimal?
4) If we give chemotherapy which agent or agents should we use and at which dose?
5) Can rates of sphincter-preserving surgery be increased by the use of adjunctive therapies?

1.8.1 Chemotherapy agents

Chemotherapy drugs inhibit tumour growth by targeting rapidly dividing cells. The evolution of chemotherapy for the treatment of colorectal and rectal cancer began with the development of 5-FU in 1957[261]. 5-FU is administered intravenously and acts by inhibiting thymidylate synthase, which in turn prevents pyrimidine nucleotide synthesis. This affects the ability of cells to synthesise DNA and leads to cell cycle

arrest and apoptosis[262]. It was established early after the development of 5-FU that it acts as a radiosensitiser[261].

Leucovorin, also known as Folinic acid, is a folic acid derivative, which is sometimes given alongside 5-FU because it potentiates 5-FU's inhibition of thymidylate synthase[262]. 5-FU has been shown to be more effective for the treatment of CRC when given via a continuous infusion rather than as a series of bolus deliveries[263]. Capecitabine is a pro-drug of fluoropyrimidine, which is given orally making the delivery of chemotherapy more convenient[210]. Capecitabine, like 5-FU, also acts as a radiosensitiser[264].

In randomised trials comparing 5-FU and capecitabine, no difference has been seen in pCR, local recurrence or survival rates[265,266]. Both 5-FU and capecitabine can cause an inflammatory skin reaction on the palms and soles, known as Palmar-Plantar Erythrodysesthesia or hand-foot syndrome; studies have shown that this occurs more frequently with capecitabine than with 5-FU[267].

A further chemotherapy drug used in rectal cancer, oxaliplatin, is a platinum agent which forms cross links in DNA, thereby preventing DNA replication, resulting in cell death[268]. Oxaliplatin acts synergistically with 5-FU and also acts as a radiosensitiser[269]. Despite its theoretical benefits, in the neoadjuvant setting, clinical trials in rectal cancer have failed to show any benefit in terms of recurrence rates or survival from adding oxaliplatin to 5-FU or capecitabine[270,271]. However, in the adjuvant setting, oxaliplatin is routinely used with capecitabine. This partly results from the MOSAIC trial, which has shown improvement in overall and disease free survival with oxaliplatin, in patients with stage III colon and rectal cancer[272]. Oxaliplatin, like other platinum agents, can lead to peripheral neuropathy and the studies have shown greater toxicity in the groups who received oxaliplatin[270-272].

Irinotecan is an inhibitor of topoisomerase I, which leads to inhibition of DNA replication and transcription[273]. Like oxaliplatin it has been shown to have theoretical benefits when given alongside 5-FU. Retrospective studies have suggested possible improvement survival benefits[274,275], however results from clinical studies have so far been disappointing[276,277]. A randomised study in the UK, the ARISTOTLE trial is aiming to definitely answer the question of whether irinotecan provides benefit, this is now completed, but not yet reported[278].

1.8.2 Radiotherapy

Radiotherapy is used to treat tumours via the delivery of ionising radiation, causing direct damage to DNA, leading to loss of reproductive capability of the cell and eventually cell death. Radiotherapy for rectal cancer has traditionally been delivered via two regimes. Short course radiotherapy (SCRT) is made up of 25 Grays (Gy) delivered in 5 fractions of 5 Gy over a one-week course. Long-course radiotherapy usually consists of 45-50 Gy delivered in 25-28 fractions; this is normally delivered over a five-week period. It is normally combined with chemotherapy and given as long course chemoradiotherapy (LCRT). There have been recent developments in radiotherapy for rectal cancer; these include reducing the field size, improvements in mapping techniques and more targeted delivery of radiation. The primary aim of these developments is to anatomically sculpt delivery of the radiotherapy dose and minimise collateral damage to surrounding tissue[279].

The response of a tumour to radiotherapy is dose dependent; ideally the dose given would be elevated to achieve maximum local control. With the external delivery of radiotherapy the dose escalation is limited because of concerns about damage to surrounding tissue[280]. Due to these limitations, the use of brachytherapy, with internal delivery of radiotherapy, has been of increasing interest in recent years. There are three methods of delivery for brachytherapy[280]:

- Contact radiotherapy, also known as Papillon
- High dose rate (HDR) intra-luminal rectal brachytherapy
- Interstitial rectal brachytherapy implant

Contact radiotherapy has a number of potential benefits. It is delivered to the tumour under direct vision and there is limited absorption by surrounding tissues. Very high doses can, therefore, be given in a short time and treatment is usually given every two weeks[280]. This technique has been used selectively for many years in patients who were unfit for surgery. More recently, the potential for adding this in to the neoadjuvant regime has been explored; this would be given as a boost to the tumour bed with the aim of increasing pCR rates.

The Lyon R96-02 trial included patients with T2 or T3 low-risk tumours, randomising them to either standard neoadjuvant radiotherapy (39 Gy in 13 fractions) vs. the same standard regime with an additional contact radiotherapy boost (85 Gy in 3 fractions). Patients in the contact radiotherapy group showed

significantly higher rates of pCR (24% vs. 2%) and of sphincter preservation (76% vs. 44%)[281]. There was no difference in local recurrence, disease-free or overall survival at 10 year follow-up[282]. Trials are currently being set up to investigate the role of contact radiotherapy in combination with TEM for early tumours, facilitating organ preservation for more patients.

1.8.3 Theoretical benefits of neoadjuvant therapy

There are a number of theoretical benefits of giving chemotherapy and/or radiotherapy pre-operatively rather than following surgery. These include:

- Treatment is delivered to intact tissue planes with an intact blood supply and therefore oxygenation optimises the susceptibility of the tumour to these therapies
- 'Sterilisation' of lymphatics within the mesorectum reducing dissemination of the tumour during dissection
- Exclusion of the small bowel from the pelvis by the rectum
- Downsizing/downstaging may improve operability of tumour
- Improved compliance with treatment
- Post-operative radiotherapy could have a greater effect on neorectal function

1.8.4 Clinical trials of neoadjuvant and adjuvant therapy in rectal cancer

Table 1.5 summarises the main trials of neoadjuvant and adjuvant therapies in rectal cancer. These are discussed in more detail below.

The first trials in this area set out to establish the role of adjuvant therapy in improving survival from rectal cancer. The Gastrointestinal Tumor Study Group carried out a 4-arm study between 1975 and 1980. Patients were randomised to surgery alone, adjuvant radiotherapy, adjuvant chemotherapy, or adjuvant chemoradiotherapy[283]. The chemoradiotherapy group showed significantly better disease-free and overall survival[283]. The National Surgical Adjuvant Breast and Bowel Project (NSABP) R-01 study then compared 3 groups: surgery alone, surgery with adjuvant radiotherapy and surgery with adjuvant chemotherapy[260]. It found that local recurrence was significantly lower in the radiotherapy group (25% vs. 16%) compared to surgery alone[260]. Disease free survival and overall survival were better in the chemotherapy group than the surgery alone group[260]. The NSABP R-02 trial then compared adjuvant chemotherapy to adjuvant chemoradiotherapy and showed

a reduction in local recurrence in the chemoradiotherapy group[284]. These studies established the important role of adjuvant therapies in improving outcomes[285].

The Swedish rectal cancer trial, which ran from 1987 to 1990, was one of the earliest randomised trials to attempt to determine the potential benefits of neoadjuvant therapy. Patients with resectable rectal cancer were randomised to pre-operative radiotherapy with 25 Gy in 5 fractions followed by surgery vs. surgery alone[286]. Pre-operative radiotherapy led to a significant reduction in local recurrence (27% vs. 11%) and a 10% improvement in 5-year overall survival (58% vs. 48%). These results were maintained in a long-term follow-up at a median of 13 years[287]. A separate analysis of late gastrointestinal effects showed that patients in the radiotherapy group had an increased risk of late small bowel obstruction and abdominal pain, and that these effects depended on the radiotherapy dose given[288].

The high recurrence rates and poor survival seen in the Swedish trial reflect its historical nature; it was conducted prior to the use of MRI for staging, with no assessment of CRM involvement and before the concept of TME surgery had been widely adopted[286]. The next study to assess the use of radiotherapy did specify (and assess) TME as the surgical approach used. The Dutch TME trial randomised patients to pre-operative radiotherapy with 25 Gy in 5 fractions followed by TME vs. TME alone[289]. The rate of local recurrence was significantly lower in the group receiving radiotherapy (2.4% vs. 8.2%)[289]. Toxicity was similar in the 2 groups although the irradiated patients who underwent APER had a higher risk of perineal complications[103]. At long-term follow up the beneficial effect of radiotherapy in reducing local recurrence was maintained but there was no difference between the groups in overall survival[293].

Table 1.5 Summary of the main trials of neoadjuvant and adjuvant therapy in rectal cancer. DFS: Disease-free survival; OS: Overall Survival; CRM: circumferential resection margin; CRT: chemoradiotherapy; RT: radiotherapy; chemo: chemotherapy; pCR: pathological complete response; Gy: Gray.

Trial	Period	Patients	TME	Treatment arms	Outcomes	Results
Gastrointestinal Tumor Study Group[283]	1975-1980	227 Dukes B2 and C	No	Surgery alone Adjuvant RT Adjuvant chemo Adjuvant CRT	DFS OS	DFS & OS improved in adjuvant CRT group
NSABP R-0[260]	1977-1986	555 Dukes B and C	No	Surgery alone Adjuvant chemo (5-FU, vincristine & semustine) Adjuvant RT	DFS OS Local recurrence	Local recurrence was lower in the RT group vs. surgery alone DFS & OS were improved in the chemo group vs. surgery alone
NSABP R-02[284]	1987-1992	694 Dukes B and C	No	Adjuvant chemo Adjuvant CRT	DFS OS Local recurrence	Local recurrence was lower in the CRT group. No difference in DFS or OS
Swedish rectal cancer trial[286]	1987-1990	1168 Resectable rectal cancer	No	Surgery alone Neoadjuvant RT (25Gy)	Mortality Local recurrence DFS, OS	Local recurrence reduced and DFS/OS improved in RT group. Mortality equivalent.
Dutch TME trial[289]	1995-1999	1861 Resectable rectal cancer	Yes	Surgery alone Neoadjuvant RT	Local recurrence OS	Local recurrence was lower in the RT group. No difference in OS
German Rectal Cancer Study Group[290]	1995-2002	823 Stage II or III	Yes	Neoadjuvant CRT Adjuvant CRT + boost RT	Local recurrence DFS, OS Sphincter preservation	Local recurrence was lower & sphincter preservation improved in the neoadjuvant CRT group. No difference in DFS/OS
Polish Rectal Cancer trial[291]	1999-2002	312 Stage II or III	Yes	Neoadjuvant CRT Neoadjuvant RT	CRM positivity, pCR, sphincter preservation, DFS, OS	CRT group had increased toxicity but lower CRM positivity rates, increased pCR. No difference in DFS, OS or sphincter preservation.
MRC CR07 [292]	1998-2005	1350 Resectable rectal cancer	No	Neoadjuvant RT Selective adjuvant CRT if CRM positive	Local recurrence DFS, OS	Local recurrence was lower and DFS better in the neoadjuvant RT group. No difference in OS

The Medical Research Council CR07 study was a randomised trial comparing short-course pre-operative radiotherapy (25 Gy in 5 fractions) with post-operative chemoradiotherapy (45 Gy in 25 fractions with concurrent 5FU) for those with a positive CRM[292]. This study showed that preoperative radiotherapy decreased local recurrence rates (4.4% vs. 10.6%) and led to increased disease free survival. There was no difference between the groups in overall survival. A later analysis looked at the effect of pre-operative SCRT on patients' quality of life. SCRT led to increased rates of male sexual dysfunction and faecal incontinence[294]. Other retrospective studies have confirmed the link between preoperative SCRT and faecal incontinence[295,296], as well as increased rates of urinary incontinence[295]. These results have led some to argue for a more selective use of preoperative radiotherapy in the UK, arguing that the CR07 was carried out before the routine use of MRI staging and the added staging information that this provides[297,298].

The German rectal cancer study was a trial randomising patients with stage II or III rectal cancer to pre-operative or post-operative chemoradiotherapy. The regime used was 50.4 Gy in 28 fractions with 5-FU; the post-operative group received the same regime with an added 5.4 Gy boost to the tumour bed[299]. Rates of local recurrence were reduced in the pre-operative group (6% vs. 13%)[299]. For patients judged at diagnosis to need an APER, the rate of sphincter preservation was more than doubled after pre-operative chemoradiotherapy[299]. Toxicity was lower and quality of life was improved in the pre-operative group[299]. There was no difference seen in disease free or overall survival[299]. At long-term follow-up there was a persisting reduction in local recurrence rates in the pre-operative group, with still no difference in survival[299]. As a result of this study, pre-operative chemoradiotherapy replaced adjuvant therapy as the standard of care for locally advanced rectal cancer; despite the details of the regime used varying from that in the study[300].

A further randomised trial carried out in Korea utilised a similar protocol for patients with stage II or III cancer, randomising to pre-operative or post-operative chemoradiotherapy with 50 Gy in 25 fractions with capecitabine rather than 5-FU. Results showed no difference between the groups in local recurrence, disease free survival or overall survival[301]. However, the primary outcome of the trial was disease free survival and it was not powered to detect a difference in local recurrence[301]. For patients with distal tumours (<5cm), those undergoing pre-operative therapy were significantly more likely to undergo a sphincter-preserving procedure[301].

There have been two randomised studies of pre-operative LCRT vs. SCRT. The Polish study randomised patients to pre-operative chemoradiotherapy with 50.4 Gy in 28 fractions with 5-FU and leucovorin followed by TME at 6-8 weeks or pre-operative radiotherapy with 25 Gy in 5 fractions followed by surgery within 7 days[291]. Chemoradiotherapy increased acute toxicity but decreased CRM involvement (4% vs. 13%) and increased complete response[291]. There was no difference seen in sphincter preservation, local recurrence and disease-free or overall survival. The trial was powered to detect a 15% difference in sphincter preservation and was therefore, once again, underpowered to detect a difference in local recurrence. There was a trend towards significance with crude incidence of local recurrence of 9.0% in the chemoradiotherapy group vs. 14.2% in the short course radiotherapy group (p=0.170) and it is possible that a larger study might have detected a difference. There was no difference seen in quality of life (measured via the validated QLQ-C30) or anorectal and sexual function (measured with an unvalidated questionnaire)[302].

The second study, conducted in Australia by the Trans-Tasman Radiation Oncology Group (TROG), randomised patients to preoperative chemoradiotherapy with 50.4 Gy in 28 fractions with 5-FU followed by surgery at 4-6 weeks or preoperative radiotherapy with 25 Gy in 5 fractions followed by early surgery[303]. Both groups also received adjuvant chemotherap[303]. The study was powered to detect a 10% difference in local recurrence at 3 years. There was no difference seen between the two groups in local recurrence, survival or toxicity[303]. However, there was once again a trend towards a difference in recurrence with 3-year rates of 4.4% in the chemoradiotherapy group vs. 7.5% in the short course radiotherapy group (p=0.24)[303]. For distal tumours <5cm, the difference in local recurrence was even greater, with 3.2% in the chemoradiotherapy group vs. 12.5% in the SCRT group (p=0.21). Criticisms were made of this study, including the fact that it was underpowered, there was a lack of inclusion of MRI staging or TME (neither of which was standard care in Australia at the time) in the protocol and no reporting of CRM positivity or the quality of mesorectal excision, making it hard to judge the surgery conducted[304].

Overall the evidence does not show a clear benefit for neoadjuvant chemoradiotherapy over SCRT, or *vice versa*. Both the studies described included patients with T3/4 'resectable' lesions; it is unclear whether these included those with an involved CRM or not; both studies were underpowered and failed to show a

meaningful difference between the two treatments. Use of chemoradiotherapy or SCRT for locally advanced rectal cancer remains variable with Northern European and Scandinavian countries using SCRT and the USA and Southern European countries preferring LCRT[305]. Within the UK the trend has been away from the use of SCRT but this depends on individual centres and does not appear to be evidence based. Theoretically some prefer LCRT as it delivers systemic chemotherapy earlier. There have also been concerns raised about operative complications following SCRT, although this has been shown to only be a problem when surgery is delayed beyond 5 days[306]. An ongoing trial may provide further evidence; the Berlin rectal cancer trial is aiming to randomise 760 patients to neoadjuvant LCRT vs. SCRT, with a primary outcome of local recurrence[307]. However, since they are including T2/3 patients there may, once again, be problems of applicability to the real-world use of these therapies.

Whether neoadjuvant therapy improves rates of sphincter preservation has been debated extensively[197]. There is no evidence that neoadjuvant short course radiotherapy can improve rates of sphincter preservation[197]. For neoadjuvant chemoradiotherapy, the situation is more complex. As mentioned above, individual trials including the German rectal cancer study have shown a benefit for neoadjuvant chemoradiotherapy in reducing the requirement for APER[290]. A number of prospective, non-randomised studies have also demonstrated rates of sphincter preservation of >70% in patients receiving neoadjuvant LCRT who were deemed to require an APER at diagnosis[308-310]. However, two meta-analyses on this topic, the first in 2006 including 10 trials and the second in 2012 including 17 trials have failed to show any improvement in rates of sphincter preservation with neoadjuvant chemoradiotherapy[311,312]. It is more likely to be the improvements in neoadjuvant therapy, in combination with the surgical developments described above, which has led to improvements in overall rates of sphincter preservation[28].

Another aspect of therapy for rectal cancer, which is under investigation, is the timing of surgery following neoadjuvant therapy. Traditionally, surgery has been timed at 6 weeks post completion of long-course radiotherapy. This was based on the results of the Lyon R90-01 trial, published in 1999, which compared intervals of 2 and 6 weeks, showing improved clinical and pathological downstaging after 6 weeks, with no increase in morbidity[313]. In recent years, a number of retrospective analyses began to establish that leaving a longer period between neoadjuvant therapy and surgery was not detrimental to outcome and that shorter periods may be linked to increased complications[314-316]. A randomised trial investigating the

possibility of lengthening this interval, the 6 vs. 12-week study led by the Royal Marsden Hospital, has shown that waiting 12 weeks leads to improved rates of downstaging and pCR[317]. These results have been presented in abstract form and are not yet formally reported. A small prospective single-arm study explored the possibility of administering further chemotherapy in the interval between neoadjuvant chemoradiotherapy and surgery, showing high tolerability and acceptable toxicity[318]. Larger prospective trials are needed to further assess this approach.

When given following surgery, adjuvant therapy most commonly uses chemotherapy, particularly 5-FU based therapy. Many studies have tried to determine the benefit of this; these have mostly concentrated on those who have not undergone neoadjuvant therapy and have had surgery alone[319]. The European Organization for Research and Treatment of Cancer (EORTC) 22921 trial was a multicentre European study, including patients with resectable T3 and T4 cancers, which compared four treatment options[320]:

- Preoperative radiotherapy;
- Preoperative chemoradiotherapy;
- Preoperative radiotherapy with adjuvant chemotherapy
- Preoperative chemoradiotherapy with adjuvant chemotherapy

This study showed no benefit for adjuvant chemotherapy on overall or disease-free survival[321] and this lack of effect remains on the latest follow-up after 10 years[322]. Despite this, the trial failed to change practice because there were problems with adherence to adjuvant chemotherapy with only 43% of patients receiving the planned dose without delay[320]. Reasons for this included post-operative complications, disease progression, patient refusal and toxicity from preoperative therapies[320]. Such problems reflect the frequent difficulties with the use of adjuvant therapy.

A Cochrane systematic review carried out in 2012 included studies randomising to adjuvant chemotherapy following surgery vs. no further treatment[319]. The meta-analysis showed that adjuvant therapy improved overall survival by 17% and disease-free survival by 25%, as well as reducing recurrence by 25%[319]. However, this review showed significant heterogeneity between the included chemotherapy regimes and did not include studies with patients who had undergone neoadjuvant

therapy, commenting that there remains a lack of evidence for adjuvant therapy in this group of patients[319].

A number of randomised trials have been established with the aim of examining the use of adjuvant therapy in patients who have had neoadjuvant therapy but have closed early due to poor accrual and failed to demonstrate any benefit. These include the high profile PROCTOR-SCRIPT and CHRONICLE studies[323,324]. Problems with the studies that have been carried out so far include slow accrual, premature closure, inadequate power and poor compliance with the treatment protocols[324]. It also appears that clinicians and patients have a lack of equipoise on this issue, which has a further negative effect on study accrual[325]. A recent systematic review has shown no benefit for the use of adjuvant therapy following preoperative CRT in terms of recurrence or survival rates[326]. The use of chemotherapy in this setting remains controversial and a recent consensus meeting to develop European guidelines for rectal cancer failed to reach consensus in this area[23], advising that decisions be made on an individual patient basis by experienced MDTs.

A number of alternative regimes for the treatment of locally advanced rectal cancer are also being investigated. The US based PROSPECT study is assessing induction chemotherapy with FOLFOX, (5-FU, oxaliplatin and leucovorin)[327]. The Dutch RAPIDO study is randomising patients to SCRT followed by pre-operative chemotherapy and then TME vs. standard chemoradiotherapy with TME[328].

1.8.5 Monoclonal antibodies

Monoclonal antibodies are a targeted biological therapy used in cancer to target antigens on cancer cells, reducing tumour growth and proliferation by a number of mechanisms including enhancing the immune system mediated destruction of cells or by blocking growth signals from the tumour. A number of these therapies have been used in the treatment of rectal cancer including cetuximab and panitumumab, which are both epidermal growth factor receptor (EGFR) inhibitors and bevacizumab, which is a vascular endothelial growth factor (VEGF) inhibitor[329]. These new therapies have been most widely explored in combination with standard chemotherapy agents for use in locally advanced and metastatic rectal cancer. Clinical use has been limited by the costs of these therapies and limitations on their use from the National Institute for Health and Care Excellence[330]. At the current time, both cetuximab and panitumumab are recommended as treatment options for metastatic colorectal cancer.

1.8.6 Complete response to neoadjuvant therapy

Surgery, with or without the use of neoadjuvant or adjuvant therapies, has always been the principle treatment for rectal cancer. As the use of neoadjuvant chemoradiotherapy has become widespread, it has been noted that a proportion of those undergoing this treatment have a complete response and when they subsequently undergo surgery there is no evidence of remaining tumour seen on histological assessment; this is known as a pathological complete response (pCR). A pooled analysis of 484 patients who had a pCR shows that these patients have improved long-term outcomes with lower recurrence and better survival[331]. These findings, in combination with the recognised morbidity and mortality risks of surgery, has led some investigators to consider the possibility of following a non-operative approach for those who appear to have had a clinical complete response (cCR).

This so-called 'watch and wait' strategy has the potential to preserve not only the sphincters but also the rectum itself. Angelita Habr-Gama and colleagues in Sao Paulo, Brazil have intensively studied this strategy. They followed a policy for many years of intensifying the neoadjuvant regime, using 50.4-54 Gy with 5-FU-based chemotherapy to try and achieve maximal rates of cCR. In their latest series, 49% of patients achieved an early cCR[332]. High local recurrence rates have always been the concern with this approach and in this series, 31% of patients developed recurrence at a median time point of 60 months[332]. The majority of these recurrences were endorectal, occurring with the lumen of the retained rectum[332]. This type of recurrence has been shown to be more amenable to curative resection than recurrence occurring outside of the rectum[333]. In keeping with this, Habr-Gama *et al.* found >90% were amenable to successful salvage surgery, with an overall local control rate of 94%[332].

The International Watch & Wait Database was established in 2014 with the aim to determine outcomes following cCR and surveillance[334]. The first report of outcomes from the database included 880 patients with a median follow-up time of 3.3 years[334]. The 2 year incidence of local recurrence was 25.2%, distant metastases occurred in 8%, 5-year overall survival was 85% and 5-year disease-specific survival was 94%[334].

A systematic review of results from all studies using this strategy included 9 studies with 650 patients but was limited in its conclusions by the lack of randomised studies

or control groups, inconsistent definition of cCR and heterogeneity in terms of the regimes used, patients included and follow-up strategies[335]. The studies identified included patients with earlier stage and smaller tumours than the majority of cancers currently treated with neoadjuvant therapy, making it unlikely that the high cCR rates seen could be reproduced in usual practice[335].

Part of the difficulty in uniting the results of studies to reach an overall assessment of outcomes is the heterogeneity of different approaches all labelled under the 'watch and wait' label. Some studies include patients who are unfit for major resection and undergo CRT with contact radiotherapy boost as the initial treatment plan; others such as Habr-Gama *et al.* have deliberately intensified the chemoradiotherapy regime, setting out to achieve maximal cCR rates and avoid surgery. A third group of, mainly retrospective, studies have collected data from patients who are given routine neoadjuvant regimes, happen to have a cCR and do not undergo surgery for a number of reasons including adverse events or patient choice[335].

Accurately assessing complete clinical response to predict those who have had pCR and facilitate a policy of 'watching and waiting' has always been a major concern regarding this strategy and has limited widespread adoption[336]. A recent review has shown that no single method can conclusively be used for assessment of cCR[336]. Multiple strategies and protocols for the follow-up of these patients have been published, utilising a variety of methods including clinical, endoscopic and imaging modalities, including ERUS, MRI, DWI and PET-CT[336]. Biopsies taken from the site of the apparently completely regressed tumour have limited value in ruling out residual cancer[337]. This has led Habr-Gama *et al*. to publish their definitions of clinical and endoscopic criteria for evaluating cCR with illustrated examples[338].

A survey of British and Irish colorectal surgeons has shown that widespread adoption of this strategy is still some way off. 58% stated they would not consider conservative management in patients with a cCR and 69% said they would not even discuss this option with a patient who was fit for resection[339]. Over 70 different combinations of investigations to define cCR were given by respondents[339].

1.8.7 Management protocols in early and locally advanced rectal cancer

Treatment protocols in rectal cancer have traditionally differentiated between early tumours and those that are locally advanced. Standard therapy for early rectal cancer has been resection with a TME followed by adjuvant chemotherapy in selected patients[38]. This remains the most frequently used treatment for these cancers. The NICE Guidelines on Colorectal Cancer published in 2011 and revised in 2014 recommend that all stage I tumours are discussed at an 'early rectal cancer' MDT and advises that patients should be informed of the lack of evidence comparing treatment strategies in these cases and offered participation in a trial where appropriate[39]. The NICE CRC treatment pathway offers the options of contact brachytherapy or surgical resection for these cancers. The most recent European consensus guidelines go further and advise options of local excision or TME for T1 lesions; advising TME as the only option for T2 cancers[340].

Standard treatment for locally advanced rectal cancer has been neoadjuvant SCRT or LCRT followed by surgery +/- adjuvant chemotherapy; this is the treatment advised by the ACPGBI 2017 Guidelines[38]. Recent guidelines reflect improvements in staging modalities, which have allowed improved differentiation between different types of locally advanced cancer. The outcomes for a T3aN0 cancer with a clear CRM and no EMVI are very different to those for a T4N2 lesion with an involved CRM and EMVI present; more recent guidelines have begun to take these considerations into account. The division of rectal cancer into three distinct risk categories has been termed 'the good, the bad and the ugly' classification, as shown in Figure 1.8[341]. Recent studies have suggested that because of resulting toxicity and lack of benefit from neoadjuvant treatment in T3a tumours where the CRM is not involved (<10% recurrence with TME alone), good outcomes may be achieved using surgery alone in selected T3N0 cases[297,342]. Similar findings have been shown on retrospective analysis of large databases[343,344].

The NICE Guidelines separate rectal cancers into low, moderate and high risk as shown in Table 1.6. Neoadjuvant therapy is not recommended for tumours in the low-risk group. SCRT is advised for those in the moderate group with LCRT used for those that are on the borderline between moderate and high and those in the high-risk group[39]. The European guidelines provide similar advice but give equal weighting to options of SCRT or LCRT for neoadjuvant therapy in T3N1-2 tumours[23].

Figure 1.8 Management according to the 'The Good, The Bad and The Ugly' classification of rectal cancer [341]. MRF: mesorectal fascia; LR: local recurrence; TME: total mesorectal excision.

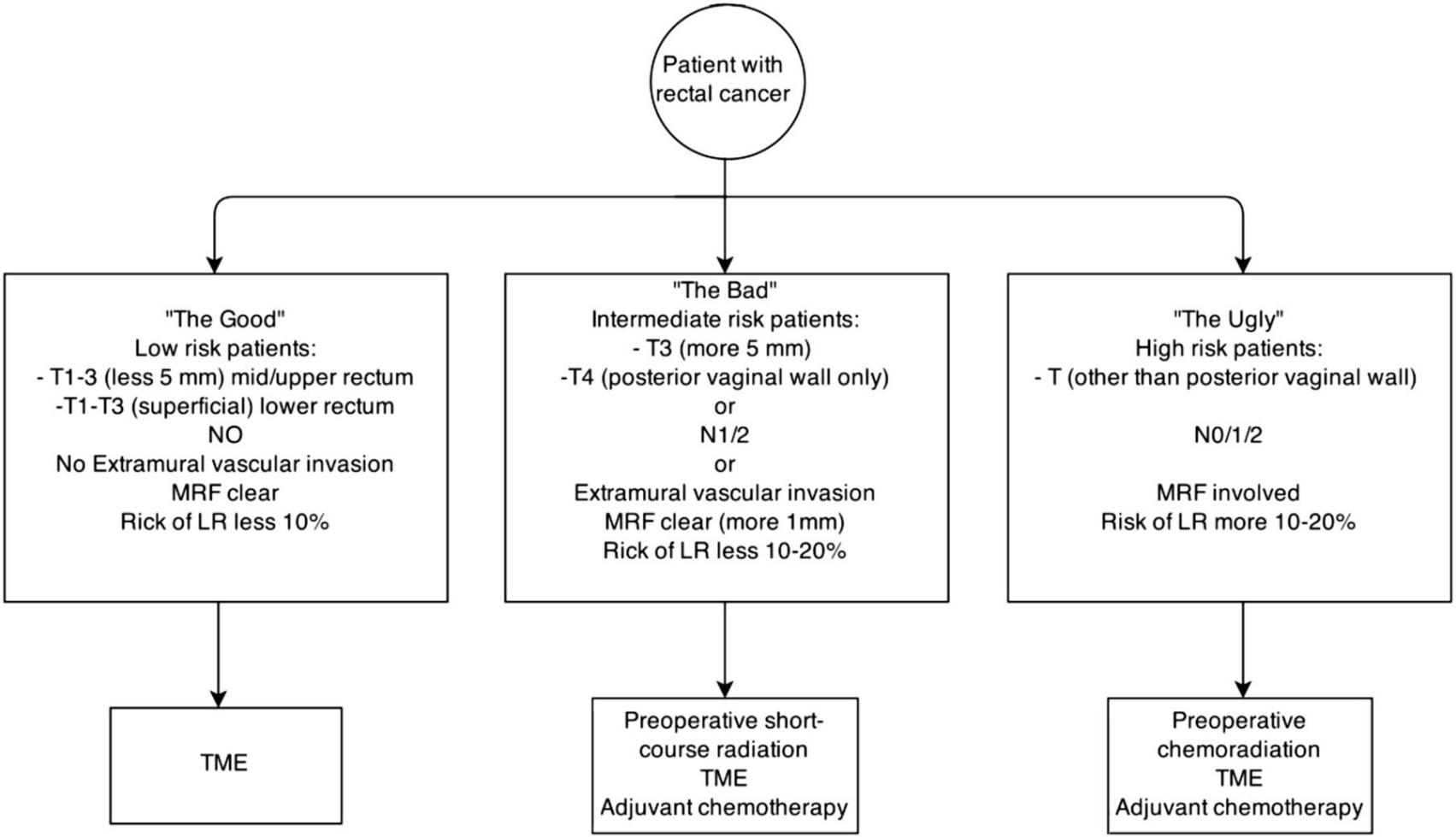


Table 1.6 Risk of local recurrence for rectal tumours as predicted by MRI. From the NICE Colorectal Cancer Pathway[39].

Risk of local recurrence	Characteristics of rectal tumours predicted by MRI
Low	• T1 or T2 or T3a and • No lymph node involvement
Moderate	• Any T3b or greater, in which the potential surgical margin is not threatened or • Any suspicious lymph node not threatening the surgical resection margin or • The presence of extramural vascular invasion
High	• A threatened (<1 mm) or breached resection margin or • Low tumours encroaching onto the inter-sphincteric plane or with levator involvement

It is likely in the future that options of dose escalation of neoadjuvant therapy with the aim of achieving cCR to facilitate organ preservation may be introduced but there is a lack of evidence for the routine use of this option. The adverse impact that dose escalation could have on the bowel function of patients who end up undergoing surgery remains unclear. Many questions remain unanswered including which patients would be chosen for this approach and how response in best assessed; as such current advice is to use this strategy only in the setting of a clinical trial[345].

1.9 Functional outcomes

It has been recognised for some time that a large proportion of patients undergoing anterior resection for rectal cancer have alteration of their bowel habit following surgery. Symptoms vary and can include incontinence, urgency, frequency, obstructed defaecation, constipation and lack of predictability[346]. This combination of symptoms following surgery has become known as 'anterior resection syndrome' (ARS)[347].

Multiple descriptions and definitions of ARS exist, but a pragmatic definition is 'disordered bowel function after rectal resection, leading to a detriment in quality of life'[348]. The different descriptions and definitions of ARS and the many different symptoms which patients experience, have led to considerable difficulties in

establishing just how common the problem is. Many small retrospective studies have assessed function following rectal resection, sometimes comparing this with functional outcomes from other procedures. The results vary considerably with the incidence of incontinence ranging from 3-79% of patients, urgency 0-69% and incomplete evacuation 2-85%[155].

These figures are taken from the only meta-analysis of long-term functional outcomes following anterior resection, the results of which were significantly limited by the heterogeneity of the primary data[155]. 65% of studies identified did not use a validated assessment tool, with lack of consistency and transparency of reporting. Inconsistencies were also noted in the prognostic factors and outcomes described and in definitions used to assess severity[155].

ARS following surgery for rectal cancer has been shown to have a considerable impact on post-operative quality of life (QOL)[349,350]. Two meta-analyses have been unable to find any difference in quality of life following anterior resection when compared with abdominoperineal excision of the rectum[351,352]. It is likely that poor function following anterior resection removes some of the benefit to patients from carrying our sphincter-preserving surgery; this needs to be better understood and predicted in order to improve individual patient counselling. A qualitative study questioning patients about their symptoms shows that they experience fear, embarrassment and vulnerability about their unpredictable bowel habit and that although ARS is a physical problem, its effects on patients are predominantly psychosocial[353].

Many studies have examined the possible causes for ARS and undoubtedly it is a multifactorial condition with a contribution from several different mechanisms[354]. These include direct damage to the anal sphincters during surgery[355,356], autonomic nerve disruption[357,358], reduced rectal reservoir[359], impaired neo-rectal compliance[360], abnormal motility of the neorectum[361], altered rectoanal inhibitory reflex[362] and a hyperactive postprandial response[363].

The configuration of the anastomosis has been considered as a possible determinant of post-operative function, with several comparisons between straight end-to-end anastomosis and colonic J-pouch shape anastomoses. A systematic review was unable to show any difference between the techniques[364], whereas a Cochrane review with meta-analysis showed that pouch configuration might provide better functional

outcome but that benefit is not likely to be maintained beyond 18 months[365]. Studies have confirmed that symptoms relate to length of time since surgery with improvement over the first year[366,367] and then stabilisation of symptoms following that. This is likely due to permanent anatomical and physiological changes[347].

Risk factors for developing ARS include neoadjuvant radiotherapy[155,368], adjuvant chemoradiotherapy[369] and the level of the anastomosis[370,371]. The impact of age on functional outcome after surgery for rectal cancer is unclear. No effect was found in the meta-analysis[155] or the one study specifically designed to answer this question[372] (although other studies have shown that age is a factor in outcome[373,374]).

The different measurement tools that have been used to assess post-operative function have led to inconsistent assessment and have not been specifically designed for LARS. Most of the scoring systems commonly in use are designed to measure incontinence and do not take into account other symptoms including urgency[375]. The authors of the meta-analysis of long-term functional outcomes, described above, criticised investigators for continually developing new scoring systems despite the existence of established validated scores, thus making reliable assessment of outcomes and comparison between populations impossible[155]. Use of a common scoring system would also facilitate testing of interventions in a meaningful way[348].

To combat these problems two validated scoring systems, specifically for ARS, have recently been developed. The Memorial Sloan-Kettering Cancer Center Bowel Function Instrument contains eighteen questions and was developed and validated in New York[376]. The Low Anterior Resection Syndrome (LARS) score contains five questions and was developed and validated by a group of researchers in Denmark[375]. It has also been validated in a European study including Swedish, Spanish and German patients[373]. This score is simple to complete, short and a high level of compliance was found during the validation studies already carried out. It is more suitable for use in clinical practice, in an outpatient setting, than the longer Memorial Sloan-Kettering score. The LARS score is ideally suited to establishing the prevalence of LARS and has recently been validated in the UK population[377].

In addition to effects on bowel function, rectal cancer surgery can have other detrimental effects for patients, including sexual dysfunction, difficulty voiding and bladder incontinence. Rates of these problems following surgery are difficult to assess as they depend on adequate recording and reporting of these problems. In a

similar way to problems with bowel function, patients may not volunteer this information unless specifically asked and it is likely that the incidence is underestimated. Urinary dysfunction with symptoms including urgency, incontinence, and incomplete emptying affects up to a third of patients after rectal resection; the main cause of this is nerve damage during surgery[378]. Reported rates of sexual dysfunction rates range from 23% to 69% in men and 19% to 62% in women[379]. During the Dutch TME trial, data on sexual dysfunction was recorded preoperatively and at each follow-up visit[378]. Overall, 76% of men and 62% of women reported either new symptoms or deterioration of pre-existing symptoms. Risk factors for sexual dysfunction included nerve damage (as recorded in operation notes), anastomotic leakage and preoperative radiotherapy[378].

1.10 Survivorship and long-term quality of life

1.10.1 Patient preferences

The priorities in the surgical management of rectal cancer are to achieve oncological clearance whilst balancing this with optimising bowel function and long-term quality of life. The views of the patient must be an important consideration during the decision-making process and some would prefer to accept a less than perfect functional outcome in order to achieve their goal of sphincter-preservation.

Current treatment strategies in rectal cancer are designed to achieve the maximal reduction in local recurrence, as documented above. When healthy volunteers were presented with data about the treatment schedule, functional and sexual outcomes following surgery alone vs. chemoradiotherapy with surgery, 54% would only accept chemoradiotherapy if it led to a 10% or greater benefit in terms of reducing the risk of local recurrence[380].

A questionnaire study looking at the beliefs of patients who have undergone surgery for rectal cancer, showed that most would be willing to accept some degree of incontinence in order to avoid an APER[381]. A further study in Australian patients looked at physician and patient preferences and found that patients' strongest preference was avoidance of a stoma and that 65% of the group would trade a mean of 34% of their remaining life expectancy to achieve this goal[382]. The views of surgeons and oncologists in this study were markedly different, in terms of preferences: surgeons appeared to be averse to radiotherapy[382]. In the same study,

when asked to choose between negative outcomes in order of acceptance, surgeons were more likely to accept a permanent colostomy than either patients or oncologists[382]. This may be because of their familiarity with stomas and their experience of patients coping with this option. This finding has implications because surgeons may underestimate the importance of this outcome to patients[382].

Part of the difficulty with these studies is that they have been carried out on patients who have already undergone treatment, which clearly affects their views on the treatments received. The team looking after them will have taken their preferences into account when deciding on the treatment options that they have received; any complications sustained will also affect their views. The importance placed on avoidance of permanent stoma; incontinence or sexual dysfunction differs greatly between individual patients and these small studies may not reflect the views of all patients. Although patients do have preferences, they often look for guidance on this complex decision from the professionals involved in their care. When asked, prior to surgery, about their preference for procedure, almost two thirds of patients would defer this decision to their surgeon, whilst 30% would opt for anterior resection and only 5% for APER[383]. One of the major difficulties in counselling patients about the likely outcomes following different surgical options is the lack of evidence about functional outcomes following surgery for rectal cancer, along with our inability to predict which patients will develop problems.

Taking into account patient preferences should be considered an integral part of the informed consent process. Following the landmark Montgomery case the guidance on informed consent has recently changed towards a patient-centred perspective with the Royal College of Surgeons reinforcing the need for surgeons to inform patients about all risks material to them and to provide information about alternative treatments[384]. This has particular relevance in rectal cancer surgery, where treatment options and pathways can be complex, and the long-term outcomes for some recent options including 'watch and wait', local excision or transanal TME surgery, may be unclear. Decisions can be difficult particularly where modest improvements in survival may potentially equate to significant effects on QOL[385].

Patients now, rightly, expect to be involved in shared decision-making[386] and this should cover potential risks, benefits and likely outcomes as well as the long-term sequelae of treatment including bowel, bladder and sexual dysfunction and their potential impact on QOL[386]. The majority of rectal cancer surgeons agree that shared

decision-making is important in rectal cancer surgery but it is still poorly utilised in practice[387]. Time pressures are one of the greatest barriers to shared decision making identified by clinicians[388] and because of this, the consent process may necessitate several discussions with a patient, and often their family, over multiple appointments. Increased discussion time has been identified as an important factor for improved comprehension[389].

Patient recall of discussions around informed consent and retention of information is poor and patients want more information on functional results and long-term QOL than clinicians may anticipate[385]. Decision support aids such as written information, videos or relevant online information may be helpful[385], but are not widely used[390]. A specific Rectal Cancer Patient Decision Aid has been shown to improve knowledge, understanding and reduce decisional conflict[391]. This may also help to adjust expectations so that patients are better able to deal with symptoms following treatment, or know where to seek help about them if they do occur.

1.10.2 Quality of life

Assessing quality of life (QOL) following surgery for rectal cancer is difficult due to a number of reasons. QOL is not only affected by the surgical treatment but by a number of other factors including other therapies and ongoing toxicity from these as well as any complications that may have arisen and the effect of any resulting further procedures. QOL is not static and is likely to change over time, being particularly affected by the time that has elapsed since treatment[392]. Individual studies have often failed to take into account the time needed for problems with bowel function to settle down following anterior resection[95]. Comparing QOL in those who have undergone anterior resection and those who have had an APER needs to take into account that the patient's preferences about these two procedures has usually played a part in decision making and patients in each group are therefore already more likely to prefer the option they have undergone. These two groups are often not comparable in terms of age, pre-operative function or tumour location in the rectum, which can all affect QOL. It is not possible to conduct a study randomising patients between APER and anterior resection because of the lack of equipoise in clinicians and patients, and the fact that permanent colostomy is generally considered an unfavourable outcome and would be avoided whenever possible. The problems that might arise when dealing with a permanent stoma are different to those of living with functional problems, and this subtlety is often missed using generic QOL scales.

Two systematic reviews of studies measuring QOL following anterior resection and APER have not shown any difference between the two groups[351,352]. The Cochrane review, published in 2012 included 35 studies, all of them were observational and non-randomised and there was also marked heterogeneity; for these reasons meta-analysis was not conducted[351]. This review suggested the need for large prospective cohort studies in which patients' QOL measurements are recorded both pre- and post-operatively[351]. An earlier systematic review, published in 2007, included 11 studies with 1443 patients in a meta-analysis and showed no difference in global health scores[352]. There were differences seen between the groups in different aspects of QOL with anterior resection patients showing higher levels of physical function and sexual function[352]. APER patients showed higher cognitive and emotional function scores[352].

1.10.3 Selection of outcomes

Making decisions about patients with rectal cancer based on evidence from available studies needs to take into account the outcomes that these studies have measured and the relevance of these. The majority of the studies described above, which comprise the current evidence base for rectal cancer treatment, have local recurrence (or measures of surgical quality used as a surrogate measure for this) as their primary end point[393]. Despite all the developments that have occurred in neoadjuvant therapy and surgical technique, leading to low levels of local recurrence, 20 to 40% of patients with rectal cancer develop metastases and ultimately die from systemic disease[393].

Overall survival is the primary aim of cancer treatments and the most important outcome for patients. Using survival as an end point for trials is problematic because the time lag between start and end of the study means that the methods tested are often out-dated by the time results are available[393]. What should replace this as the end point in clinical studies is not an easy decision. As described above, what matters most to clinicians and patients is not always the same. Surgical trials may use a range of end points such as downstaging, TRG or even pCR rates, which although measurable, have little meaning to the lives of the patients treated. Other outcomes, which are often not well measured, may be important to patients, these include overall quality of life, functional outcomes, and toxicities resulting from therapy as well as the emotional and financial costs of treatment.

A recent survey of CRC survivors in England set out to use patient reported outcome measures (PROMs) for the first time in a National study to capture the patient experience. The tools used included the EQ-5D quality of life measure, FACT Items (Functional Assessment of Cancer Therapy) and SDI (Social Difficulties Inventory)[394]. This identified a wide range of lifestyle issues affecting the patients including problems with appetite, tiredness, reduced sleep, embarrassment, social distress, financial concerns, difficulty travelling, mood swings, difficulty concentrating and fear of cancer returning[394]. 'Frequently occurring challenges' identified by patients included[394]:

- The emotional impact of cancer and treatment
- On-going social and financial problems
- Long-term and age-related illnesses that could exacerbate, or be exacerbated by, problems associated with cancer treatment
- Unpleasant physical side-effects of treatment

This study has shown the wide range of effects that cancer and its treatments can have on patients. These outcomes have, in the past, been poorly recorded and viewed as having secondary importance to clinically defined outcomes such as recurrence. PROMs are becoming increasingly recognised within the NHS as a vital tool to measure the impact that treatment is having on patients. It is likely that future studies will be required to include this type of instrument in addition to traditionally used end points.

1.11 Conclusions

Achieving the optimum oncological outcome is the primary aim of rectal cancer treatment. Maximising rates of sphincter preservation is also a key goal but it is meaningless if the functional outcomes are poor, affecting quality of life. What we ideally need to achieve is maximal rates of functioning sphincters wherever this is possible. Surgically, the limits of what is technically feasible continue to expand. Patient preferences are important but it is crucial to ensure that patients are counselled appropriately, and have realistic and accurate expectations of whatever treatment choice is decided on[95].

Over-treatment and under-treatment of rectal cancer can both have detrimental effects for patients[395]; unfortunately a judgement as to whether either of these has

occurred can usually only be made at the completion of therapy. Failing to achieve adequate local control of disease can have a detrimental effect on survival and clearly this needs to be avoided. On an individual level, patients might therefore be willing to accept over-treatment to ensure survival, opting to deal with the later consequences of this once they are through the initial treatment. When the whole population of rectal cancer patients is considered, over-treatment becomes a problem; as more people live for many years following treatment it is important that treatment for cancer does not lead to detrimental effects on long-term QOL.

The range of options for therapy in rectal cancer is broad and multifaceted; it is likely to keep expanding. All of this means increasingly complex discussions with patients and often their family members. This involves different members of the MDT and usually needs multiple discussions, with time for the patient to consider information between them. There is a balance to be reached between achieving treatment in a timely way to permit maximum benefit whilst allowing the patient the time necessary to negotiate the decisions that need to be made, often based on incomplete evidence about the likely outcomes.

It is increasingly likely that in the future the focus will move away from achieving maximal rates of sphincter preservation and towards achieving organ preservation by avoiding resection of the rectum for as many patients with rectal cancer as possible. Although treatment protocols are useful for guiding therapy decisions; ultimately these need to be made based on individual patient circumstances and preferences; the goal in the future would be for them to also be based on individual tumour biology.

1.12 References

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Chapter 2. Aims and objectives

2.1 Research question

The introductory chapter has outlined the progress that has been made over recent years in the delivery of treatment for rectal cancer, with developments in many areas including neoadjuvant therapy and minimally invasive surgery. Treatment pathways for rectal cancer are complex and involve the whole multidisciplinary team. The developments that have been made have focussed on improving oncological outcomes but there has been comparatively little interest in functional outcomes.

Across the whole of cancer care, there has been a move towards recognition of the importance of patient centred outcomes, and an emphasis on survivorship. Clinicians and researchers have recognised the potential impact that cancer therapies can have on long-term quality of life and begun to explore ways to limit the long-term negative impacts of cancer treatment. For rectal cancer specifically, there has been a clear paradigm shift in the last few years. The focus is no longer necessarily on sphincter preservation, but on preservation of acceptable anorectal function.

The overall research question asked in this thesis is:

- What determines the ability to preserve functioning sphincters during management of low rectal cancer?

This overall research question can be broken down into these further questions:

- How many patients have a poor functional outcome and what determines this?
- Can we find biomarkers to help us rationalise treatments that we know have a potential negative impact on functional outcome?
- What tools can help us to understand the physiological changes that underlie poor functional outcomes?

We currently have only an estimate of the number of patients who develop Low Anterior Resection Syndrome (LARS), which indicates a poor functional outcome following anterior resection (the current gold standard surgical option for rectal cancer). In order to improve functional outcomes, we need to know how many patients are affected by LARS. We also need to better understand the factors that might determine whether a patient develops LARS.

Neoadjuvant chemoradiotherapy is known to be a major risk factor for poor functional outcome. Response to this treatment is variable and we cannot currently predict this on an individual level. It is possible that we could limit the negative effects by using it only in those patients who would respond to it and derive oncological benefit from this treatment. The ability to predict who would respond would allow us to stratify and individualise care but in order to achieve this predictive biomarkers are needed.

Anorectal physiology has been studied for many years using standard anorectal manometry. Despite this, we do not fully understand the alterations in structure or function of the anorectum following rectal cancer treatment and why some patients that develop these changes experience LARS and others are asymptomatic. High resolution anorectal manometry (HRAM) is a newly developed investigative method that converts manometry readings into colour contour pressure topography plots, this allows appreciation of the anorectum as a functional unit and can show dynamic changes. The potential for HRAM as a tool to better understand the physiological changes underlying functional change following treatment for rectal cancer has not yet been explored.

2.2 Aims and objectives

The overall aim of thesis was to explore the factors that determine the ability to preserve functioning sphincters during management of low rectal cancer. The following specific objectives will be met by each chapter:

1. To determine the proportion of patients having undergone anterior resection in the UK who have LARS (chapter 3).
2. To identify risk factors for LARS and to confirm the link between LARS and quality of life (chapter 3).
3. To determine the potential ability of measures derived from diffusion weighted MRI to predict and assess response to neoadjuvant LCRT for rectal cancer (chapter 4).
4. To identify microRNA targets with potential use as predictive biomarkers of response or non-response to neoadjuvant CRT in rectal cancer (chapter 5).
5. To define the changes in anal sphincter function following anterior resection and chemoradiotherapy using HRAM (chapter 6).

Chapter 3. Anterior resection syndrome following sphincter-preserving resection in the UK population

3.1 Introduction

Over the last 25 years, developments in the treatment of rectal cancer, outlined in the introductory chapter, have led to dramatically improved outcomes and, in particular, improved survival. Overall 5-year survival from rectal cancer has now reached 54%, with 10-year survival being only slightly lower[1]. Similar improvements in care have also been seen in many other cancers. This rise in the number of survivors has led to a change in emphasis in cancer therapy and improved awareness that these patients will need to live with the long-term effects of their treatments. Survivors from colorectal cancer have identified unpleasant physical side-effects from their treatment as a particular problem[2].

In the early years following the advent of sphincter preserving surgery for rectal cancer, there was already interest in the functional outcomes for patients[3]. Investigations into physiological changes following rectal resection began during the 1980s[4]. From the 1990s onwards the term "anterior resection syndrome" (ARS) became widely used to describe dysfunction following anterior resection[5]. Alternatives to anterior resection, particularly those that facilitate resection of very low rectal cancers, have been shown to have an even greater effect on long-term function[6].

Anterior resection syndrome can be defined as "disordered bowel function after rectal resection, leading to a detriment in quality of life"[7]. Symptoms include flatus and faecal incontinence, altered frequency, urgency, clustering, fragmentation and evacuatory dysfunction[7,8]. It essentially leads to unpredictable bowel habit, which can cause distress and feelings of vulnerability[9]. Although it is a physical condition, the effects are frequently psychosocial and have a detrimental impact on quality of life[8,10]. Rectal cancer survivors are more likely than those surviving from other colorectal cancers to suffer from 'social distress' experiencing difficulties relating to family, social activities, finances and work[2].

Anterior resection syndrome is a complex multifactorial condition with different mechanisms contributing in individual patients. The physiological reasons that have been proposed for these symptoms are discussed in more detail in chapter 6.

Intuitively it makes sense that loss of the rectum, with its sophisticated ability to control defaecation would lead to dysfunction. This is partly confirmed by the finding that those undergoing partial mesorectal excision (PME) with retention of the most distal rectum have less likelihood of developing symptoms than those undergoing TME with the entire rectum removed[11].

Several risk factors have been identified for ARS including neoadjuvant radiotherapy, height of anastomosis, total as opposed to partial mesorectal excision and younger age[11-13]. Other risk factors have been suggested by smaller studies but have yet to be confirmed, including temporary stoma[14], anastomotic configuration[15] and obstructive presenting symptoms[16]. The main limiting factor in determination of risk factors is that they overlap and confound each other. For example, a patient with an advanced tumour is more likely to undergo neoadjuvant therapy, more likely to have a technically challenging operation and more likely to require a defunctioning stoma. This makes analysis of the contributing factors more difficult.

It has long been recognised that following anterior resection, bowel related symptoms are most troubling during the initial 12-month period, this has been confirmed by a number of studies[17,18]. However, it was previously believed that with additional time post surgery, symptoms would continue to improve and this has recently been questioned[11,13]. The symptoms of ARS are now considered to be long lasting and reflect the permanent changes in anatomy and physiology following surgery[19]. Detailed physiological studies have suggested that the situation may be more complicated with symptoms of urgency and tenesmus improving as compliance improves, but incontinence worsening with time as the rectoanal inhibitory reflex returns[20].

There has been uncertainty about the proportion of patients that suffer from these problems for many years. A meta-analysis of long-term functional outcomes following anterior resection, published in 2011 showed that results in individual studies vary considerably[21]. This is shown by the very wide range of proportions reported by primary studies within this analysis, for example the proportion of those with incontinence ranged from 3-79% and urgency 0-69%. Further analysis was done to determine the cause of the variation. The authors found significant heterogeneity of the primary data; 65% of studies identified did not use a validated assessment tool, with lack of consistency and transparency of reporting. The pooled proportions suffering from each symptom were as follows: incontinence 35%; incomplete

evacuation 55%; urgency 35% and clustering 59%, indicating that a considerable percentage of patients will experience one or more symptoms.

The Scheer meta-analysis also found that 65% of the studies identified did not use a validated assessment tool to measure symptoms. Many of the studies that have assessed functional outcomes have utilised study groups from other trials, for example the Dutch TME trial, and have not, therefore, been designed to assess functional outcomes[22]. Other studies have used non-validated tools[18,23] and where they have used validated tools, these have been designed to solely measure incontinence e.g. the Wexner score, and do not include other symptoms[24,25].

The Low Anterior Resection Syndrome (LARS) score was developed to resolve the lack of measurement tool. It contains five questions and is easily completed in clinical practice, showing a high level of patient compliance[26]. The original development of the score was carried out in Denmark[26] and it was validated in a European study including Swedish, Spanish and German patients[27]. It has subsequently been validated in the UK population[28]. The LARS score has also been used, by the same study group, to assess quality of life (QOL) in the Danish population[8]. Results from the validation studies suggest LARS is a much greater clinical problem than anticipated previously[12] but prevalence in the UK population has not been independently verified.

Over recent years, there has been an uptake of minimally invasive surgery in the UK with 58% of colorectal cancer resections now carried out laparoscopically[29]. As outlined in the introductory chapter, there is conflicting evidence about the oncological long-term outcomes following laparoscopic vs. open surgery. Similarly, there is a lack of evidence about the likely impact laparoscopic surgery will have on functional outcomes and the possible effect of further developments including robotic surgery and transanal approaches. There is also an increasing recognition of the potential effect that the sequelae of cancer treatments can have on patients' long-term quality of life[2]. All these developments make this is an ideal time for an exploration of the impact of current practice on functional outcomes following rectal cancer surgery in the UK.

3.2 Aims

The primary aim of this study was to determine the proportion of patients having undergone anterior resection in the UK who have anterior resection syndrome.

The secondary aims were to identify risk factors for anterior resection syndrome and to determine the link between LARS and QOL.

3.3 Methods

3.3.1 Study design

This was a UK based epidemiological questionnaire-based cohort study. The study had a single site, Queen Mary University London, but used multiple Participant Identification Centres (PICs) to identify eligible participants.

3.3.2 Outcomes

The primary outcome was the proportion of participants with no LARS (score 0-20 on LARS questionnaire), minor LARS (score 21-29) and major LARS (score 30-42).

The secondary outcomes were the relationship between LARS and QOL and the risk factors for LARS including: gender, age (above and below median), neoadjuvant therapy and stoma use.

3.3.3 Participant Identification Centres

Invitation letters were sent to 40 NHS Hospital Trusts in the UK. Letters were addressed to Consultant Colorectal Surgeons via their work email addresses. PICs identified eligible participants using records from colorectal cancer multi-disciplinary team meetings, audit data from the National Bowel Cancer Audit Project or other local prospective databases. Participants were identified by their own direct care team only, allowing confidentiality to be maintained. Figure 3.1 lists the PICs involved in the study.

Figure 3.1 Participant Identification Centres (abbreviations in brackets used in subsequent tables)

- Barking, Havering & Redbridge University Hospitals (BHR)
- Barts Health NHS Trust (Barts)
- Brighton and Sussex University Hospitals NHS Trust (Brighton)
- Colchester Hospital University NHS Foundation Trust (Colchester)
- Guy's and St Thomas' NHS Foundation Trust (GSTT)
- Homerton University Hospital NHS Foundation Trust (Homerton)
- London North West Healthcare NHS Trust (St Marks)
- Maidstone & Tunbridge Wells NHS Trust (M&TW)
- NHS Fife (Fife)
- NHS Forth Valley (Forth)
- NHS Grampian (Grampian)
- NHS Greater Glasgow and Clyde (Glasgow)
- NHS Highland (Highland)
- NHS Lothian (Lothian)
- NHS Tayside (Tayside)
- Nottingham University Hospitals NHS Trust (NUH)
- Royal Devon & Exeter NHS Foundation Trust (RD&E)
- Royal Free London NHS Foundation Trust (Barnet)
- United Lincolnshire Hospitals NHS Trust (ULH)

3.3.4 Study population

Adult patients who had undergone anterior resection for rectal cancer at least 12 months previously were eligible to participate. If they had a defunctioning stoma at the time of surgery, this must have been reversed at least 12 months previously.

3.3.5 Inclusion criteria

Inclusion criteria were as follows:

- Age ≥ 18 years
- Anterior resection for rectal cancer and ≥ 12 months post surgery
- If defunctioning stoma used, ≥ 12 months post reversal

3.3.6 Exclusion criteria

Exclusion criteria were as follows:

- Any inclusion criteria not met
- Presence of a stoma
- Local recurrence of cancer
- Current adjuvant therapy
- Further rectal surgery after the initial operation
- Insufficient written English to participate

3.3.7 Data collection

PICs were sent pre-packed and stamped envelopes containing a letter introducing the study (Appendix 1), a participant information sheet (Appendix 2); the study questionnaire (Appendix 3) and a prepaid envelope in which to return the questionnaire. PICs then addressed these using the details of eligible participants and sent them out to their home address. Eligible participants completed the anonymous questionnaire, which was returned in the prepaid envelope directly to the study team at Queen Mary University London. Data were entered into a specifically designed database. 5% of the data were rechecked by an independent person to ensure accuracy of data entry and no errors were identified.

3.3.8 Questionnaire

Part 1 - Demographic and clinical information

Questions in part 1 confirmed eligibility to participate and assessed risk factors for LARS. The first two questions recorded the participants' age and gender. Further questions were related to eligibility, including confirmation that the patient had undergone surgery for rectal cancer, the date of the surgery, stoma formation (if applicable) and reversal date, further surgery and ongoing treatment. The remainder of the questions were about possible risk factors including: details of neoadjuvant and adjuvant therapy, as well as mode of surgery. As this part of the questionnaire was unvalidated, it was trialled with members of the public confirming that the language was understandable.

Part 2 - LARS questionnaire

This has five questions about bowel function and scores from 0–42. Results are classified into three categories: no LARS (0-20); minor LARS (21-29) and major LARS (30-42). Figure 3.2 shows the LARS questionnaire including the scoring method.

Figure 3.2 LARS questionnaire with scoring [26]

Q1. Do you ever have occasions when you cannot control your flatus (wind)?	
– No, never	0
– Yes, less than once per week	4
– Yes, at least once per week	7
Q2. Do you ever have any accidental leakage of liquid stool?	
– No, never	0
– Yes, less than once per week	3
– Yes, at least once per week	3
Q3. How often do you open your bowels?	
– More than 7 times per day (24 hours)	4
– 4-7 times per day (24 hours)	2
– 1-3 times per day (24 hours)	0
– Less than once per day (24 hours)	5
Q4. Do you ever have to open your bowels again within one hour of the last bowel opening?	
– No, never	0
– Yes, less than once per week	9
– Yes, at least once per week	11
Q5. Do you ever have such a strong urge to open your bowels that you have to rush to the toilet?	
– No, never	0
– Yes, less than once per week	11
– Yes, at least once per week	16

Part 3 - EORTC QLQ-C30

Part 3 of the questionnaire was the European Organization for Research and Treatment of Cancer (EORTC) generic quality of life questionnaire QLQ-C30[30]. This is a validated, openly available, questionnaire, which asks 30 short questions about health to determine quality of life. The scores produce:

- One global QOL scale
- Five functional scales: physical functioning, role functioning, emotional functioning, cognitive functioning and social functioning

- Nine symptom scales: fatigue, nausea and vomiting, pain, dyspnoea, insomnia, appetite loss, constipation, diarrhoea and financial difficulties.

3.3.9 Statistical analysis

The LARS questionnaire was scored according to the published scoring system (see Figure 3.2). The QLQ-C30 questionnaire was scored according to the scoring manual from EORTC[31]. Details of scoring for the questionnaires were not seen by the participants.

Incomplete questionnaires were not included in the final analysis. Questionnaires were counted as incomplete if:

- More than one part of the clinical details had been left empty
- More than one part of the LARS questionnaire had been left empty
- The whole QLQ-C30 questionnaire was empty

Single missing answers for the QLQ-C30 were scored according to the guidance in the scoring manual for dealing with missing answers. Single missing answers for the LARS questionnaire were given the minimum score for that category.

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS Inc. Chicago, USA, version 22.0) and SAS software (SAS Institute, Marlow, UK, version 9.4). A two-sided p value of <0.05 was considered statistically significant. Descriptive statistics including mean, median, percentages, range, interquartile range and odds ratios (OR) with 95% confidence intervals (CI) are reported. No adjustments were made for multiplicity of testing.

Risk factors for LARS were treated as dichotomous variables. Age was taken as ≤ or > median age. Time since surgery was taken as ≤ or > median years. Similarly, length of time with stoma was taken as ≤ or > time in months. Initial analyses (see results section) using the Chi-square test showed no difference in outcomes between short-course radiotherapy (SCRT) and long-course chemoradiotherapy (LCRT). Neoadjuvant radiotherapy was therefore dichotomised into none vs. SCRT/LCRT. Mode of operation was dichotomised as open/converted to open vs. laparoscopic surgery.

Univariable and multivariable analyses were carried out to identify potential risk factors for the following binary groups:

- No LARS vs. minor/major LARS
- No LARS/minor LARS vs. major LARS
- No LARS vs. major LARS

Multivariable analysis was carried out using a forward stepwise approach.

A multivariable ordinal regression model was fitted using no LARS, minor LARS and major LARS groups as the response variable. Forward selection was used to identify a subset of key significant univariate risk factors. Analyses were carried out twice, including questionnaires with one missing LARS score and then excluding these questionnaires.

Data from the QLQ-C30 questionnaire are reported using means, as this is standard practice[32], and compared using the Kruskal-Wallis test as scores have skewed distributions[33]. Values for population data are taken from the EORTC QLQ-C30 Reference Values document and are the values provided for the general population[34]. Quality of life results for respondents were compared with results for the general population using a one-sample T test. QOL results between the different LARS categories were analysed using Goodman and Kruskal's gamma for ordinal data.

3.3.10 Ethical arrangements and research governance

The study was sponsored by Queen Mary University London. The National Research Ethics Service (NRES) Committee East Midlands - Derby approved the study on 07/03/2014 (Reference: 14/EM/0117) (Appendix 4). The study was registered with the ClinicalTrials.gov Protocol Registration System on 14/08/2014 (Identifier number: NCT02190656). Approval was gained from the Research & Development departments at each individual PIC.

Written informed consent was not obtained. The information sheet explained the study including that participants were free to choose whether to participate. Completion and return of the questionnaire was therefore taken as implicit consent.

3.4 Results

3.4.1 Response rates

19 NHS Hospital Trusts (24 hospitals) acted as PICs to identify 2275 eligible participants between July 2014 and September 2015. One participant self-identified as eligible by contacting the study team directly, she had seen the details of the study on the ClinicalTrials.gov website. A total of 1197 replies were received, giving an overall response rate of 53%.

The flowchart in Figure 3.3 shows the reasons for ineligibility, 86 respondents were categorised as ineligible. A further 18 of the questionnaires were not included as they were incomplete. In total, 1093 questionnaires were included in the analysis. Table 3.1 shows the response rate at each of the PICs.

Figure 3.3 Flowchart showing number of respondents and final numbers included in analysis. LARS: low anterior resection syndrome; QOL: quality of life.

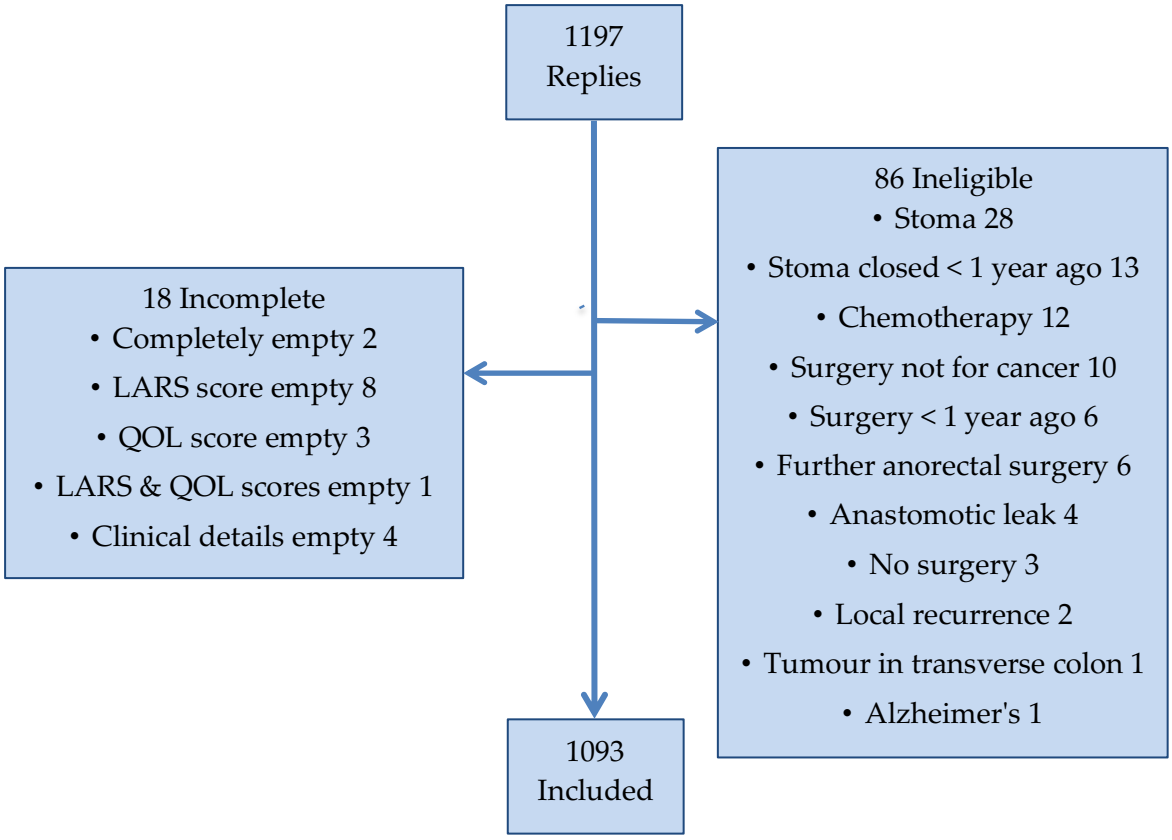


Table 3.1 Response rates by PIC. For full names of PICs see Figure 3.1.

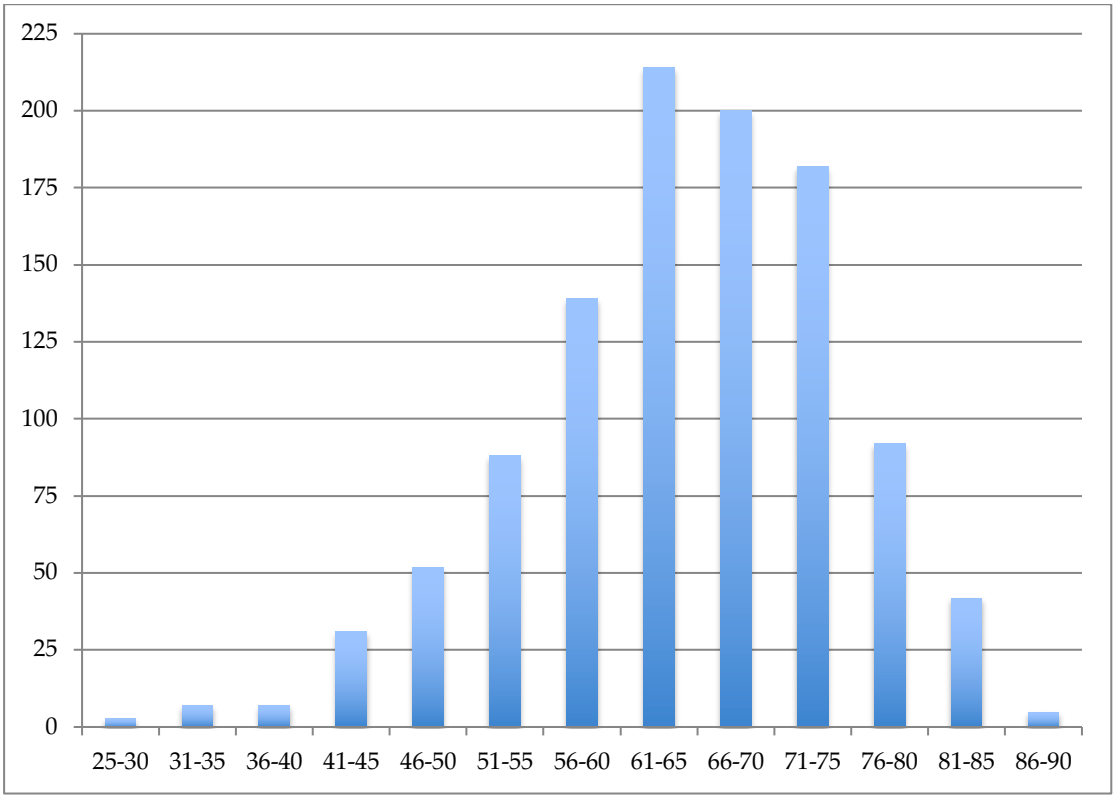
	Sent	Responded	Response rate (%)	Incomplete	Ineligible	Included
BHR	88	43	48.9	0	1	42
Barts	284	106	37.3	4	4	98
Brighton	200	107	53.5	0	19	88
Colchester	50	39	78.0	0	1	38
GSTT	26	6	23.1	0	1	5
Homerton	38	14	36.8	0	0	14
St Marks	147	66	44.9	1	4	61
M&TW	173	115	66.5	2	1	112
Fife	91	47	51.6	0	4	43
Forth	20	12	60.0	1	1	10
Grampian	54	30	55.6	0	0	30
Glasgow	66	31	47.0	1	1	29
Highland	75	51	68.0	0	3	48
Lothian	490	239	48.8	8	11	220
Tayside	116	70	60.3	0	13	57
NUH	113	67	59.3	0	3	64
RD&E	81	58	71.6	1	5	52
Barnet	37	17	45.9	0	0	17
ULH	125	78	62.4	0	14	64
Patient	1	1	100	0	0	1
Overall	2275	1197	52.6	18	86	1093

3.4.2 Participants

Overall 64% of the respondents were male and 36% female. 31 respondents did not provide their age. The median age of the respondents was 65 with an interquartile range (IQR) of 59-72 and range of 28-88. Figure 3.4 shows the breakdown of respondents into five-year age groups.

The year in which the respondents underwent surgery ranged from 1990 to 2014; 27 respondents did not answer this question. 90% of respondents had undergone surgery in the five years preceding the study. The median number of years since surgery was 3 with an IQR of 2-4.

Figure 3.4 Number of respondents by age category



3.4.3 Proportion of participants with defunctioning stoma

559 (51%) respondents had a defunctioning stoma created at the time of surgery. This result varied considerably by Trust, with a range of 10-100%. The median length of time before closure of a defunctioning stoma was 7 months with an IQR of 5-12 months. The shortest time to reversal was 2 weeks; the longest was 47 months. Table 3.2 shows the proportion undergoing defunctioning stoma in each NHS Trust and median length of time to closure.

The risk factors for taking longer than median time to stoma closure on multivariable analysis were adjuvant chemotherapy OR 6.109 (95% CI 4.047 – 9.221, p = <0.001) and neoadjuvant radiotherapy OR 1.764 (95% CI 1.021 – 3.046, p = 0.042). Age, gender, neoadjuvant chemotherapy and mode of operation were not significant. The median length of time taken to stoma closure in those undergoing adjuvant chemotherapy was 11 months compared with 6 months in those not having adjuvant chemotherapy.

Table 3.2 Use of defunctioning stoma and length of time with stoma by PIC. For full names of PICs see Figure 3.1.

	Number with stoma	% with stoma	Median number of months with stoma	IQR months with stoma
BHR	25	59.5	12	7-14
Barts	63	64.3	9	7-12
Brighton	25	28.4	12	7-13
Colchester	30	78.9	4	3-8
GSTT	4	80.0	5	3-9
Homerton	10	71.4	12	8-12
St Marks	37	60.7	7	5-12
M&TW	71	63.4	6	3-11
Fife	9	20.9	9	5-12
Forth	1	10.0	2	-
Grampian	17	56.7	6	5-9
Glasgow	25	86.2	5	3-6
Highland	32	66.7	7	4-10
Lothian	63	28.6	7	4-9
Tayside	14	24.6	8	4-9
NUH	27	42.2	9	6-10
RD&E	38	73.1	6	5-8
Barnet	17	100.0	6	5-12
ULH	51	79.7	12	7-14
Patient	0	0	-	-
Overall	559	51.1	7	5-12

The risk factors for stoma formation are shown in Table 3.3 below. Neoadjuvant radiotherapy had the highest odds ratio for stoma formation on multivariable analysis; the percentage with a stoma was 90% in those having neoadjuvant radiotherapy compared with 39% in those who did not.

Table 3.3 Risk factors for stoma formation.
CI: confidence intervals; NS: not significant; lap: laparoscopic.

	No stoma	Stoma	Univariable odds ratio (95% CI)	p-value	Multivariable odds ratio (95% CI)	p-value
Gender • Female • Male	 220 313	 174 385	 1 1.555 (1.213 – 1.994)	 <0.001	 1 1.532 (1.153 – 2.035)	 0.003
Age • ≤65 • >65	 267 251	 274 270	 1 1.048 (0.824 – 1.333	 0.701	 NS	 0.122
Neoadjuvant chemotherapy • No • Yes	 501 30	 388 165	 1 7.102 (4.709 – 10.712)	 <0.001	 NS	 0.252
Neoadjuvant radiotherapy • No • Yes	 507 24	 329 228	 1 14.640 (9.401 – 22.797)	 <0.001	 1 10.908 (6.245 - 19.054)	 <0.001
Mode of operation • Lap • Open/ converted	 249 283	 169 385	 1 2.004 (1.564 – 2.570	 <0.001	 1 1.483 (1.123 – 1.960)	 0.005

3.4.4 Reoperations and ongoing treatment

240 (22%) of patients had undergone 284 reoperations since their anterior resection; 206 had 1 subsequent operation; 24 had undergone 2 and 10 patients had 3 reoperations. Of the 284 reoperations, 24% were for metastatic disease, with 42 liver and 26 lung resections. 23% of reoperations were for complications of the anterior resection, the details of these are given below. The remaining 53% of reoperations were unrelated surgery, the most frequent being groin hernias and joint replacements.

The following reoperations were carried out for complications of anterior resection:

- 26 incisional hernia repairs
- 11 stoma site hernia repairs
- 10 laparotomy for adhesions
- 9 wound repair/drainage of wound infection
- 2 drainage of post-operative collection
- 4 surgery for ileostomy reversal complication
- 2 excision of scar tissue
- 2 operations to remove retained swab

3.4.5 Use of neoadjuvant and adjuvant therapy

The proportion of patients undergoing neoadjuvant short-course radiotherapy (SCRT) and long-course chemoradiotherapy (LCRT) was variable between different Trusts, these results are also shown in Table 3.4. Overall 23.2% underwent neoadjuvant radiotherapy of some form.

The likelihood of having neoadjuvant radiotherapy was significantly higher in those aged ≤65 years with 26.9% having radiotherapy, compared with 19.9% in those >65 years (OR 1.475, 95% CI 1.107 – 1.967, p = 0.008). There was no significant difference in the proportion undergoing neoadjuvant radiotherapy by gender. The percentage specifically undergoing SCRT or LCRT was not dependent on age or gender.

A low percentage of patients, 2.7% underwent neoadjuvant chemotherapy without radiotherapy and a much higher percentage, 39.5% had adjuvant chemotherapy. The proportion undergoing adjuvant chemotherapy did not depend on gender but was dependent on the age of the patient. 46.5% of those aged ≤65 years had adjuvant chemotherapy, compared with 31.9% of those >65 (OR 1.858, 95% CI 1.446 – 2.387, p = <0.001).

4.5% of respondents had previously undergone radiotherapy for a different indication, the majority (43%) had this for breast cancer but others had undergone pelvic radiotherapy, particularly for prostate cancer, which may affect bowel function.

Table 3.4 Use of neoadjuvant and adjuvant therapy by NHS Trust. For full names of PICs see Figure 3.1; SCRT: short-course radiotherapy; LCRT: long-course chemoradiotherapy.

NHS Trust	Neoadjuvant and adjuvant therapy - % respondents			
	Neoadjuvant therapy			
	Chemotherapy alone	SCRT	LCRT	Adjuvant chemotherapy
BHR	0	12.2	56.1	53.7
Barts	4.1	6.1	29.6	52.0
Brighton	5.7	2.3	4.5	19.3
Colchester	0	0	10.5	28.9
GSTT	20.0	0	0	60.0
Homerton	0	14.3	21.4	57.1
St Marks	4.9	0	8.2	36.1
M&TW	4.5	11.7	11.7	41.4
Fife	2.3	7.0	0	34.9
Forth	10.0	0	0	30.0
Grampian	6.7	13.3	43.3	46.7
Glasgow	3.4	0	55.2	17.2
Highland	0	2.1	29.2	39.6
Lothian	1.4	7.8	7.3	43.8
Tayside	1.8	0	0	49.1
NUH	1.6	7.8	7.8	40.6
RD&E	0	0	5.8	28.8
Barnet	0	5.9	35.3	35.3
ULH	1.6	30.2	31.7	36.5
Patient	0	0	0	0
Overall	**2.7**	**7.2**	**16.0**	**39.5**

3.4.6 Mode of surgery

Overall 38.5% of patients had their surgery carried out laparoscopically, as with other results this proportion showed wide variation between different NHS Trusts, from 7.1 to 75.4%. Table 3.5 shows results for mode of operation for each NHS Trust. The rate of conversion from laparoscopic to open surgery also varied widely from 0 – 21.4%.

On multivariable analysis, the only risk factor for open surgery (compared with those that started laparoscopically including the converted cases) was having undergone neoadjuvant radiotherapy (OR 2.630, 95% CI 1.714 – 4.037, p = <0.001). Conversion from laparoscopic to open operation was independent of age, gender and neoadjuvant chemotherapy.

Table 3.5 Mode of operation for each NHS Trust. For full PIC names see Figure 3.1.

NHS Trust	Mode of operation - % respondents		
	Open	Converted	Laparoscopic
BHR	52.4	7.1	40.5
Barts	39.2	12.4	48.5
Brighton	30.7	14.8	54.5
Colchester	13.2	18.4	68.4
GSTT	40.0	0	60.0
Homerton	71.4	21.4	7.1
St Marks	18.0	6.6	75.4
M&TW	50.9	9.8	39.3
Fife	48.8	16.3	34.9
Forth	70.0	0	30.0
Grampian	76.7	10.0	13.3
Glasgow	71.4	10.7	17.9
Highland	70.2	8.5	21.3
Lothian	62.8	8.7	28.4
Tayside	40.4	8.8	50.9
NUH	73.4	4.7	21.9
RD&E	45.1	13.7	41.2
Barnet	35.3	11.8	52.9
ULH	65.1	14.3	20.6
Patient	0	0	100
Overall	**50.9**	**10.6**	**38.5**

3.4.7 Bowel function and LARS score

Overall 37% of respondents had no LARS, 22% had minor LARS and 41% major LARS. The proportion of respondents in each LARS category for the different NHS Trusts is shown in Table 3.6. The mean LARS score overall was 24. Only 75 respondents (7%) had a LARS score of 0.

69.7% respondents experienced flatus incontinence (19.6% less than once each week and 50.1% more than once weekly), compared with 46.5% experiencing liquid incontinence (27.9% less than once weekly and 18.6% more than once weekly). Clustering was the most frequently occurring symptom, affecting 78.8% of respondents. Figure 3.5 shows the breakdown of LARS symptoms for all respondents with Figure 3.6 showing each symptom by those in the different LARS categories. Figure 3.7 shows the results for frequency of bowel movements, for all patients and for each LARS category.

Table 3.6 Respondents in each LARS category for each NHS Trust. For full names of PICs see Figure 3.1. LARS: low anterior resection syndrome.

	No LARS (n)	No LARS (%)	Minor LARS (n)	Minor LARS (%)	Major LARS (n)	Major LARS (%)
BHR	11	26.2	10	23.8	21	50.0
Barts	35	35.7	13	13.3	50	51.0
Brighton	42	47.7	17	19.3	29	33.0
Colchester	7	18.4	11	28.9	20	52.6
GSTT	1	20.0	3	60.0	1	20.0
Homerton	4	28.6	5	35.7	5	35.7
St Marks	20	32.8	12	19.7	29	47.5
M&TW	51	45.5	20	17.9	41	36.6
Fife	15	34.9	8	18.6	20	46.5
Forth	6	60.0	3	30.0	1	10.0
Grampian	12	40.0	10	33.3	8	26.7
Glasgow	4	13.8	6	20.7	19	65.5
Highland	22	45.8	8	16.7	18	37.5
Lothian	89	40.5	49	22.3	82	37.3
Tayside	21	36.8	16	28.1	20	35.1
NUH	29	45.3	13	20.3	22	34.4
RD&E	17	32.7	16	30.8	19	36.5
Barnet	4	23.5	3	17.6	10	58.8
ULH	18	28.1	15	23.4	31	48.4
Patient	0	0	0	0	1	100.0
Overall	**408**	**37.3**	**238**	**21.8**	**447**	**40.9**

Figure 3.5 Frequency of component symptoms for LARS (all respondents)

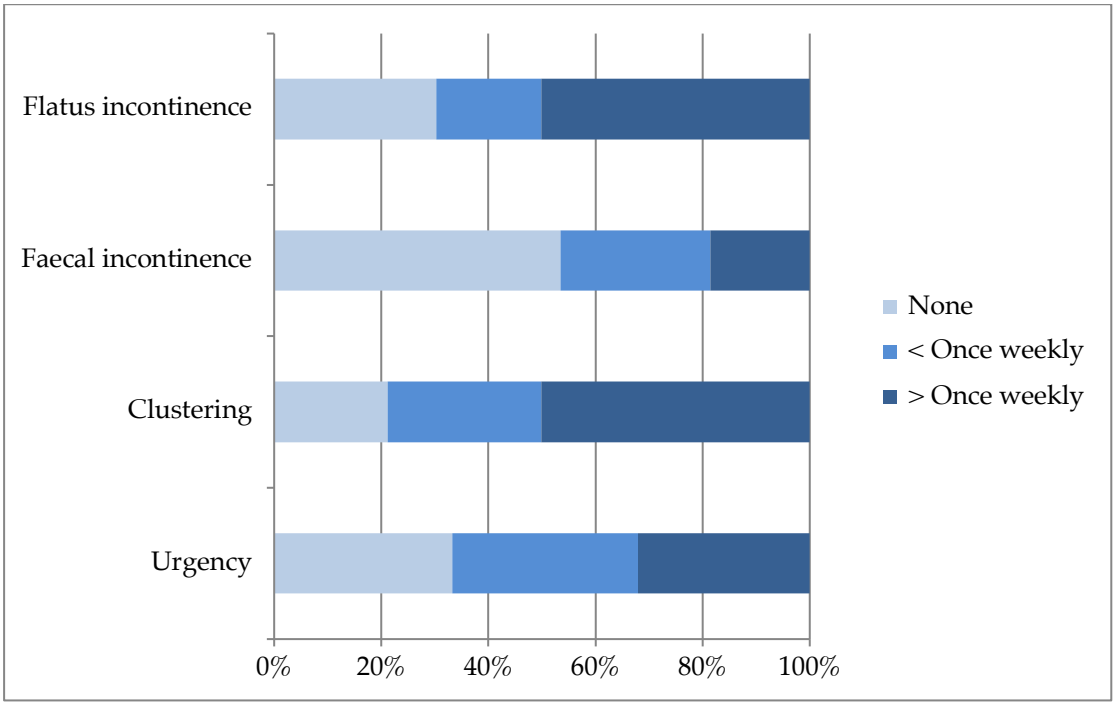


Figure 3.6 Frequency of component symptoms in the different LARS categories. LARS: low anterior resection syndrome.

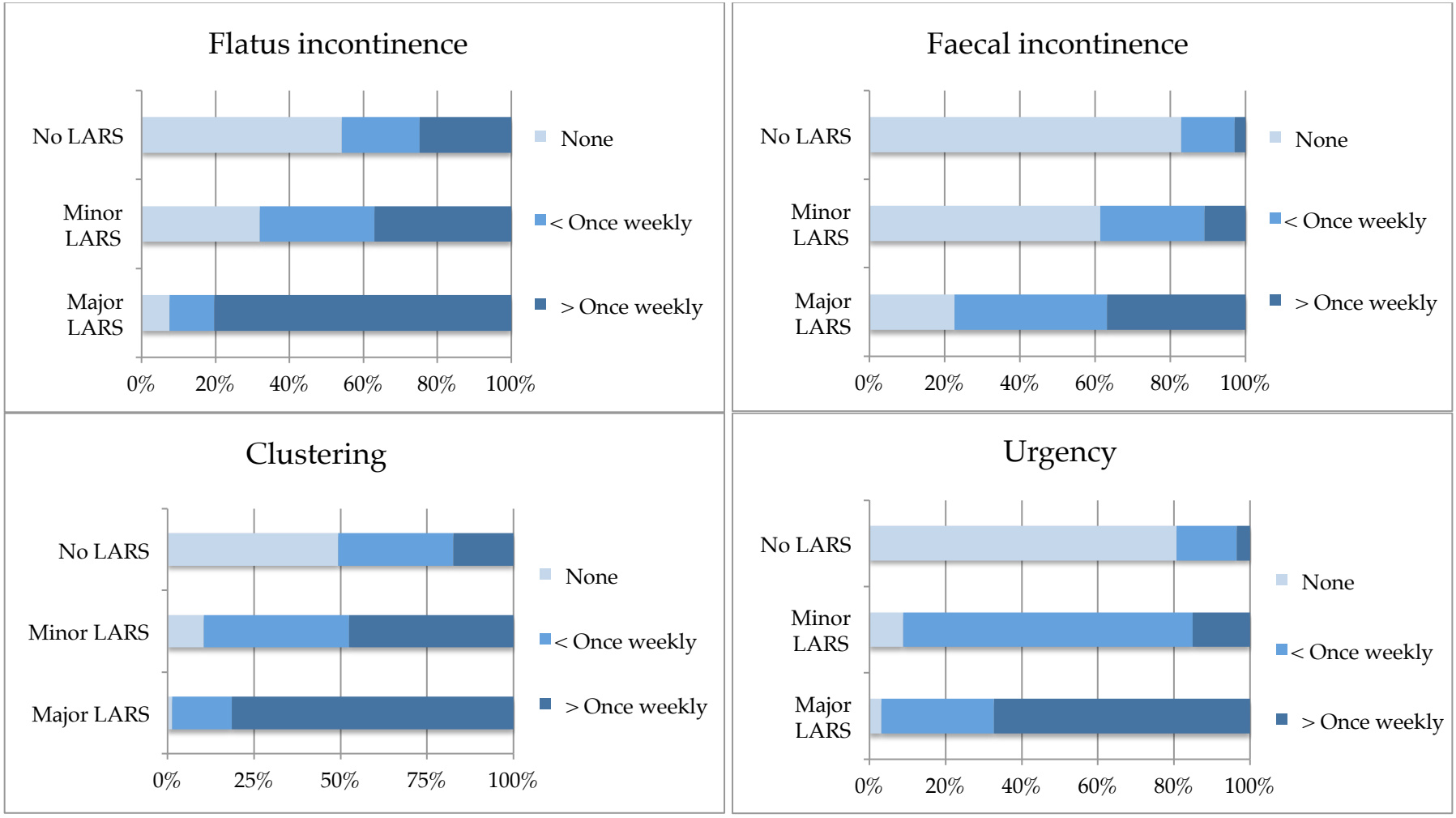
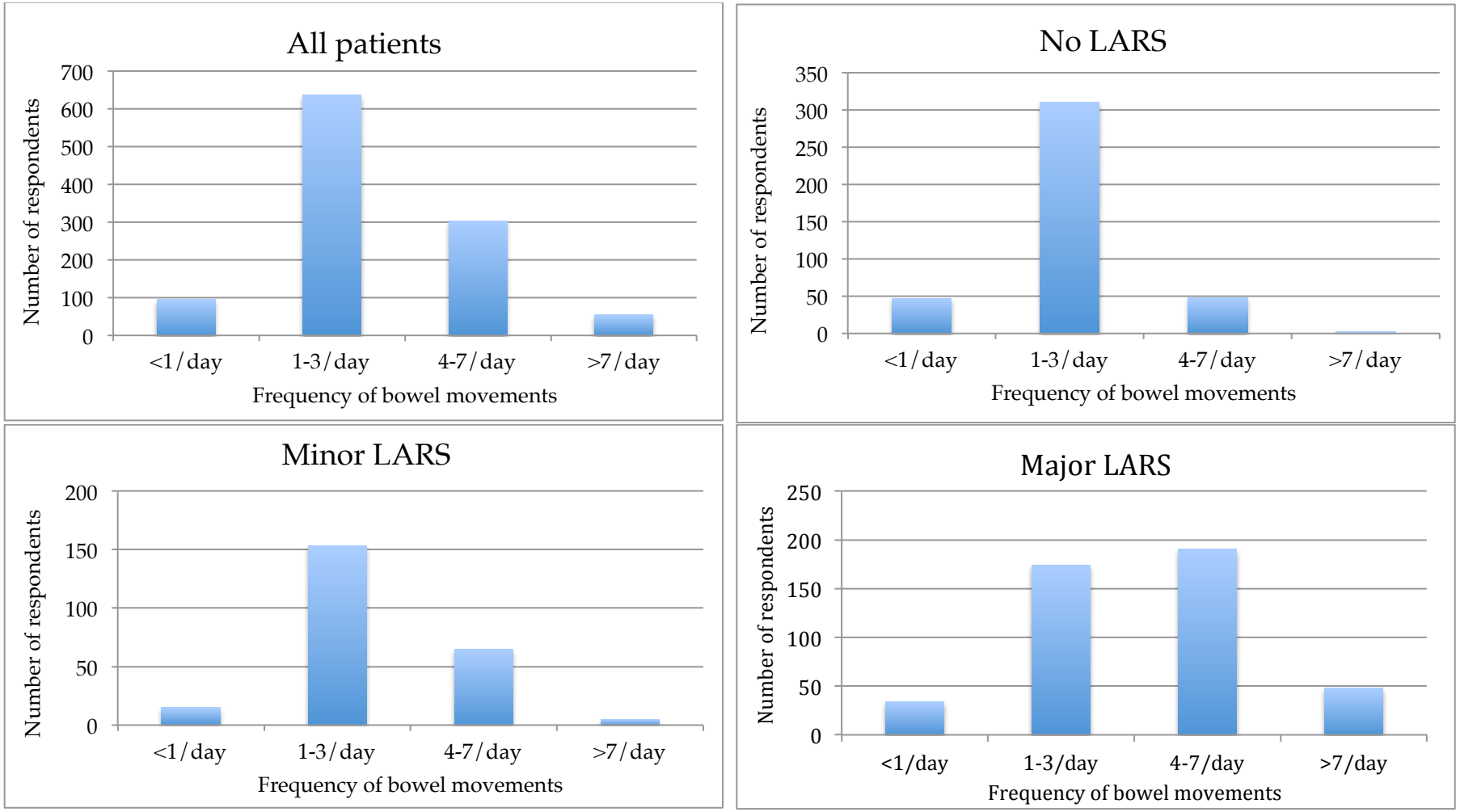


Figure 3.7 Frequency of bowel movements for all respondents and by LARS category. LARS: low anterior resection syndrome.



3.4.8 Risk factors for LARS

There was no difference between those who had undergone SCRT and LCRT in the proportion with no LARS, minor LARS or major LARS (χ^2 (2) = 0.284; p = 0.868). Following this finding, the binary outcome of neoadjuvant radiotherapy (including both SCRT and LCRT) was used in further analysis. The risk factors for LARS on univariable analysis are shown in Table 3.7. The results for multivariable binary logistic regression are shown in Table 3.8 and those for ordinal stepwise logistic regression are in Table 3.9. The ordinal regression proportional odds assumption was met (χ^2 (7) = 8.55; p = 0.286). As shown in Table 3.9, the significant risk factors for LARS are: age ≤65 years (OR 1.46, 95% CI 1.16 – 1.84; p = 0.002); defunctioning stoma (OR 2.59, 95% CI 2.00 – 3.36; p = 0.001); neoadjuvant radiotherapy (OR 1.67, 95% CI 1.22 – 2.27, p = 0.002) and female gender (OR 1.54, 95% CI 1.20 – 1.96; p = 0.001).

The same risk factors were found on multivariable binary logistic regression, see Table 3.8. There was very little difference between results with missing values included and excluded, or between the different binary combinations of LARS categories (Table 3.8). The only difference was that for major LARS vs. minor/no LARS with missing values excluded, age was no longer a significant risk factor. The proportion of those correctly allocated into a LARS category using the risk factors as a model was also consistent across the different binary combinations and with or without missing values included, ranging from 65.0 to 66.1%

With none of the significant risk factors present, in respondents who are male, >65 years, with no stoma and no radiotherapy 61.0% have no LARS, 16.3% minor LARS and 22.7% major LARS. With all four risk factors present, female, ≤65 years, stoma and radiotherapy 15.9% have no LARS, 20.5% minor LARS and 63.6% have major LARS.

Figure 3.8 shows box and whisker plots for LARS scores in different groups according to the risk factors included in analysis. p-values shown were calculated using the Mann Whitney test. The median LARS score for those undergoing radiotherapy was 32 compared with 24 in those who did not (p = <0.001); median score was 30 in those who had defunctioning stoma vs. 20 in those without (p = <0.001).

As shown above, neoadjuvant radiotherapy is a risk factor for LARS. 57.5% of those undergoing radiotherapy developed major LARS, with 22.2% having minor LARS and only 20.2% no LARS, vs. 35.9% major LARS, 21.4% minor LARS and 42.7% no LARS in those who did not have radiotherapy. Figure 3.9 and 3.10 show the symptom breakdown for those with neoadjuvant radiotherapy and without. Those who have had radiotherapy were significantly more likely to experience symptoms of LARS: flatus incontinence, faecal incontinence, urgency and clustering were all more likely ($p = <0.001$ for each with Mann Whitney test). Clustering was the most frequently experienced symptom by those with and without radiotherapy (90.9 vs. 75.1%). Only 40.9% of those who had undergone radiotherapy opened their bowels 1-3 times/day compared with 63.6% of those who had not had radiotherapy. 6 patients had radiotherapy with no symptoms of LARS (0.6% of all patients and 2.4% of those who had radiotherapy), compared with 69 patients (%) who did not have radiotherapy (6.3% of all patients and 8.3% of those who did not have radiotherapy).

The likelihood of LARS did not depend on length of time since surgery; no significant difference in major LARS vs. no LARS was shown between those who were less than, or more than, 3 years since surgery (>3 years OR 0.892, 95% CI 0678 – 1.173, p-value 0.413). Figure 3.11 shows the breakdown of LARS category for those in the first seven years since surgery (further years were not included as there were very small numbers in each year).

Table 3.7 LARS risk factors on univariable analysis. 95% CI = 95% confidence intervals. * = significant at p <0.05. LARS: low anterior resection syndrome.

	Major vs. minor/no LARS				Major vs. no LARS				Major/minor vs. No LARS			
	Missing values included		Missing values excluded		Missing values included		Missing values excluded		Missing values included		Missing values excluded	
Variables	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Female gender	1.314 (1.023 - 1.688)	0.032*	1.288 (1.000 - 1.660)	0.050	1.333 (1.008 - 1.764)	0.044*	1.315 (0.990 - 1.747)	0.059	1.225 (0.947 - 1.585)	0.123	1.220 (0.940 - 1.584)	0.134
Age category ≤65 years	1.321 (1.034 - 1.688)	0.026*	1.291 (1.007 - 1.655)	0.044*	1.552 (1.181 - 2.040)	0.002*	1.514 (1.148 - 1.996)	0.003*	1.553 (1.209 - 1.994)	0.001*	1.525 (1.185 - 1.964)	0.001*
Years since operation ≤3	1.155 (0.903 - 1.477)	0.252	1.149 (0.895 – 1.475)	0.275	1.121 (0.853 – 1.474)	0.413	1.112 (0.842 – 1.467)	0.455	1.048 (0.816 – 1.346)	0.712	1.038 (0.806 – 1.337)	0.772
Stoma	2.718 (2.116 - 3.491)	<0.001*	2.724 (2.115 - 3.509)	<0.001*	3.682 (2.775 - 4.885)	<0.001*	3.679 (2.763 - 4.898)	<0.001*	3.067 (2.374 - 3.962)	<0.001*	3.053 (2.357 - 3.957)	<0.001*
Neoadjuvant radiotherapy	2.421 (1.817 - 3.226)	<0.001*	2.479 (1.852 - 3.319)	<0.001*	3.383 (2.374 - 4.821)	<0.001*	3.471 (2.421 - 4.976)	<0.001*	2.937 (2.098 - 4.112)	<0.001*	2.991 (2.124 - 4.213)	<0.001*
Neoadjuvant chemotherapy	2.086 (1.525 - 2.854)	<0.001*	2.184 (1.588 - 3.004)	<0.001*	2.662 (1.820 - 3.893)	<0.001*	2.859 (1.935 - 4.223)	<0.001*	2.346 (1.635 - 3.368)	<0.001*	2.505 (1.728 - 3.632)	<0.001*
Adjuvant chemotherapy	1.047 (0.818 - 1.340)	0.715	1.039 (0.809 - 1.334)	0.766	0.958 (0.729 - 1.259)	0.757	0.948 (0.719 - 1.250)	0.703	0.893 (0.695 - 1.147)	0.377	0.884 (0.687 - 1.139)	0.342
Mode: open/ converted (vs. laparoscopic)	1.286 (1.001 - 1.652)	0.049*	1.248 (0.968 - 1.609)	0.087	1.344 (1.018 - 1.774)	0.037*	1.314 (0.992 - 1.741)	0.057	1.263 (0.982 - 1.624)	0.069	1.254 (0.972 - 1.617)	0.082

Table 3.8 LARS risk factors on multivariable binary logistic regression. ns: not significant; 95% CI: confidence intervals; LARS: low anterior resection syndrome. * = significant at p <0.05.

	Major vs. minor/no LARS				**Major vs. no LARS**				**Major/minor vs. No LARS**			
	Missing values included		Missing values excluded		Missing values included		Missing values excluded		Missing values included		Missing values excluded	
Variables	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Female gender	1.559 (1.190 - 2.042)	0.001*	1.541 (1.172 - 2.025)	0.002*	1.660 (1.216 - 2.266)	0.001*	1.656 (1.207 - 2.272)	0.002*	1.495 (1.129 - 1.979)	0.005*	1.500 (1.129 - 1.994)	0.005*
Age category ≤65 years	1.304 (1.006 - 1.689)	0.045*	ns	ns	1.530 (1.138 - 2.058)	0.005*	1.504 (1.115 - 2.031)	0.008*	1.569 (1.203 - 2.047)	0.001*	1.549 (1.184 - 2.026)	0.001*
Stoma	2.475 (1.862 - 3.289)	<0.001*	2.419 (1.815 - 3.222)	<0.001*	3.181 (2.308 - 4.384)	<0.001*	3.160 (2.284 - 4.372)	<0.001*	2.704 (2.023 - 3.615)	<0.001*	2.686 (2.002 - 3.603)	<0.001*
Neoadjuvant radiotherapy	1.664 (1.198 - 2.311)	0.002*	1.757 (1.262 - 2.444)	0.001*	2.132 (1.417 - 3.207)	<0.001*	2.222 (1.467 - 3.366)	<0.001*	1.950 (1.323 - 2.874)	0.001*	2.022 (1.363 - 3.000)	<0.001*
Neoadjuvant chemotherapy	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Adjuvant chemotherapy	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Years since operation >3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mode: open/ converted (vs. laparoscopic)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Percentage correctly classified by model	65.0%		65.0%		66.1%		66.1%		65.2%		65.0%	

Table 3.9 LARS risk factors on multivariable ordinal stepwise logistic regression. Missing values were included for this analysis. ns: not significant; CI = confidence intervals; LARS: low anterior resection syndrome. * = significant at p <0.05.

	No LARS	Minor LARS	Major LARS	Multivariable odds ratio (95% CI)	p-value
Gender					
Missing (n=1)					
Male	272	157	269	Reference	0.001*
Female	135	81	178	1.54 (1.20 – 1.96)	
Age					
Missing (n=31)					
≤ 65	175	125	241	1.46 (1.16 – 1.84)	0.002*
>65	222	102	197	Reference	
Stoma					
Missing (n=0)					
No	269	111	154	Reference	0.001*
Yes	139	127	293	2.59 (2.00 – 3.36)	
Neoadjuvant radiotherapy					
Missing (n=5)					
No	357	179	300	Reference	0.002*
Yes	51	56	145	1.67 (1.22 – 2.27)	
Adjuvant chemotherapy					
Missing (n=4)					
No	240	152	267	NS	NS
Yes	168	83	179		
Years since operation					
Missing (n=27)					
≤ 3 years	220	122	255	NS	NS
> 3 years	178	107	184		
Mode of surgery					
Missing (n=7)					
Open/converted	235	145	288	NS	NS
Laparoscopic	170	93	155		

Figure 3.8 LARS scores for different risk factors. Box and whisker plots showing differences between LARS scores for: age category, gender, stoma, neoadjuvant radiotherapy, adjuvant chemotherapy and years since surgery. The middle line in each box represents the median value, with the boundaries of the box representing the quartiles and whiskers showing minimum and maximum values. LARS: low anterior resection syndrome; op: operation.

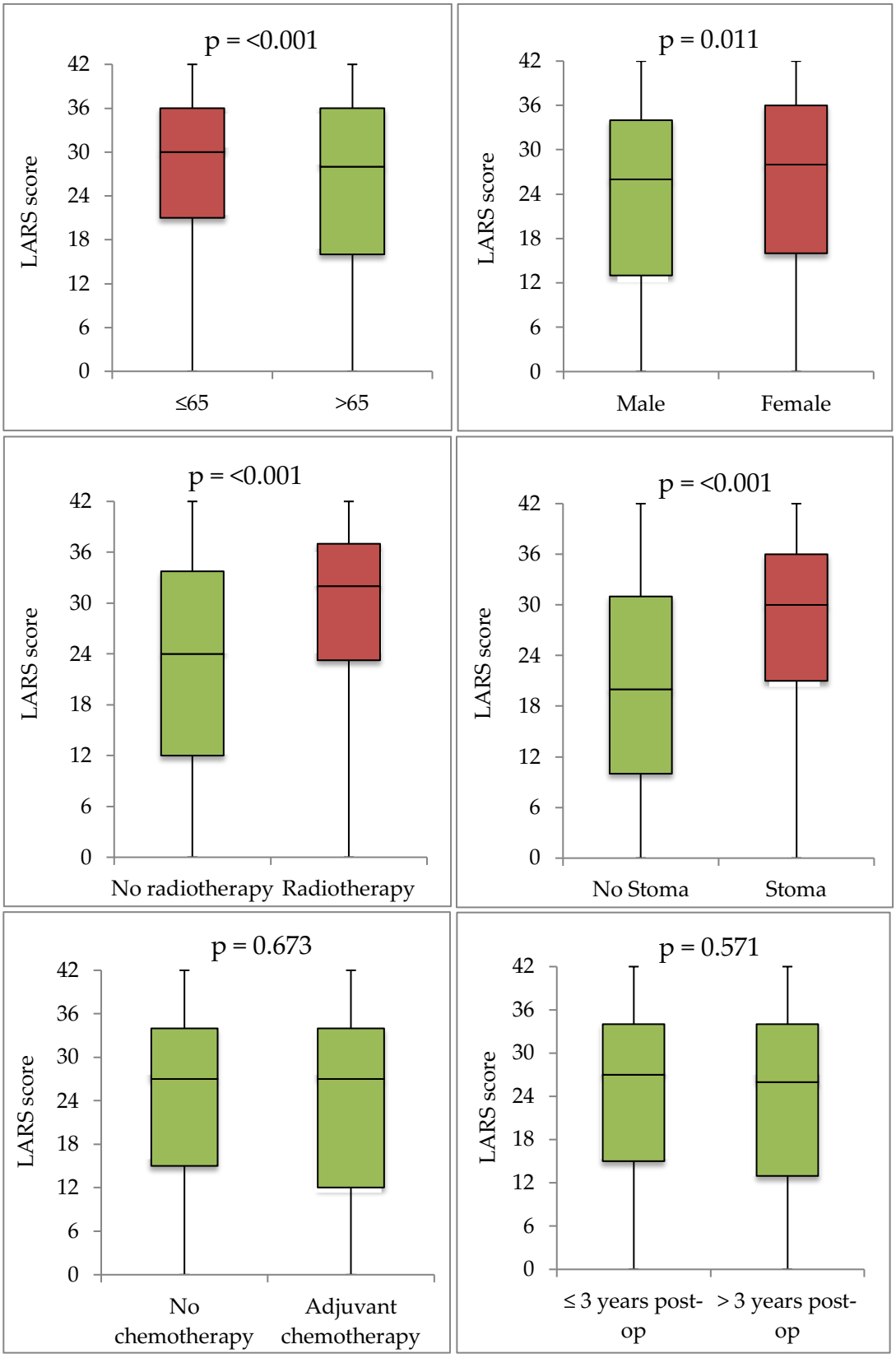


Figure 3.9 Frequency of component symptoms with and without radiotherapy

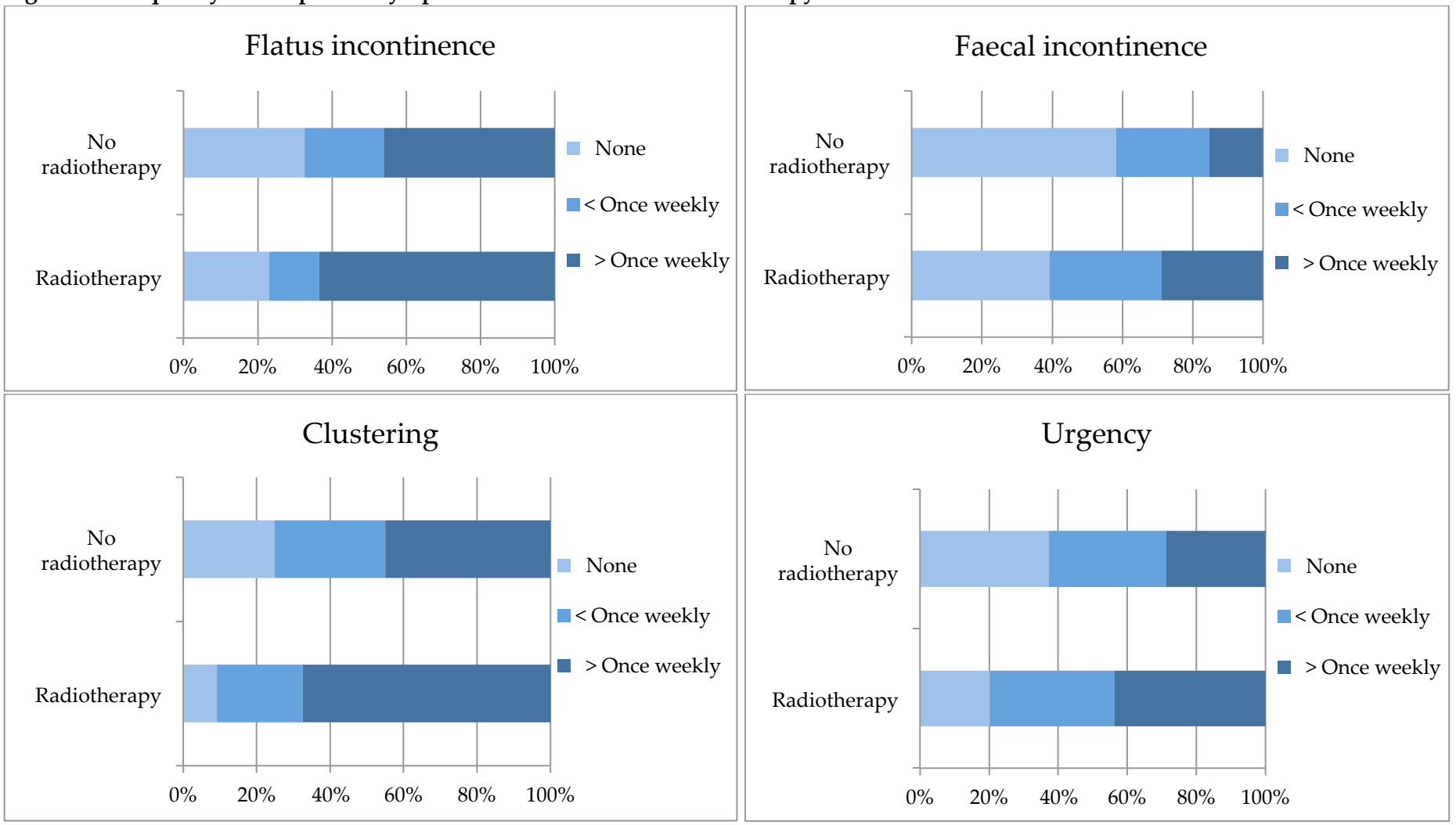
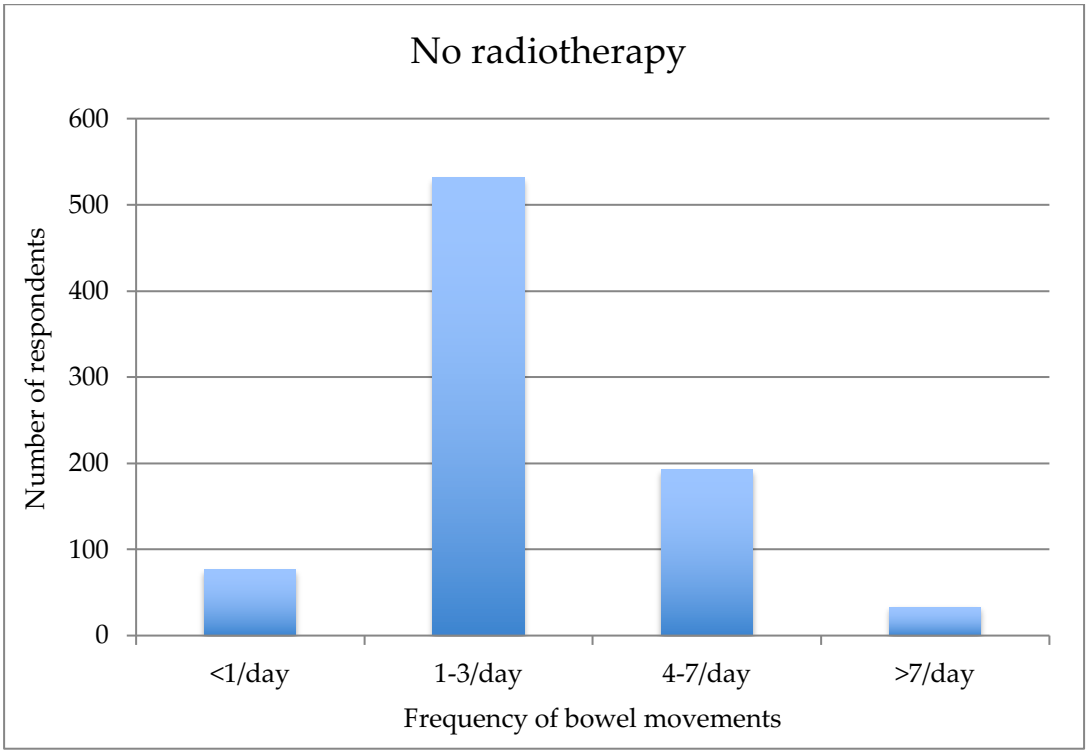


Figure 3.10 Frequency of bowel opening with and without radiotherapy



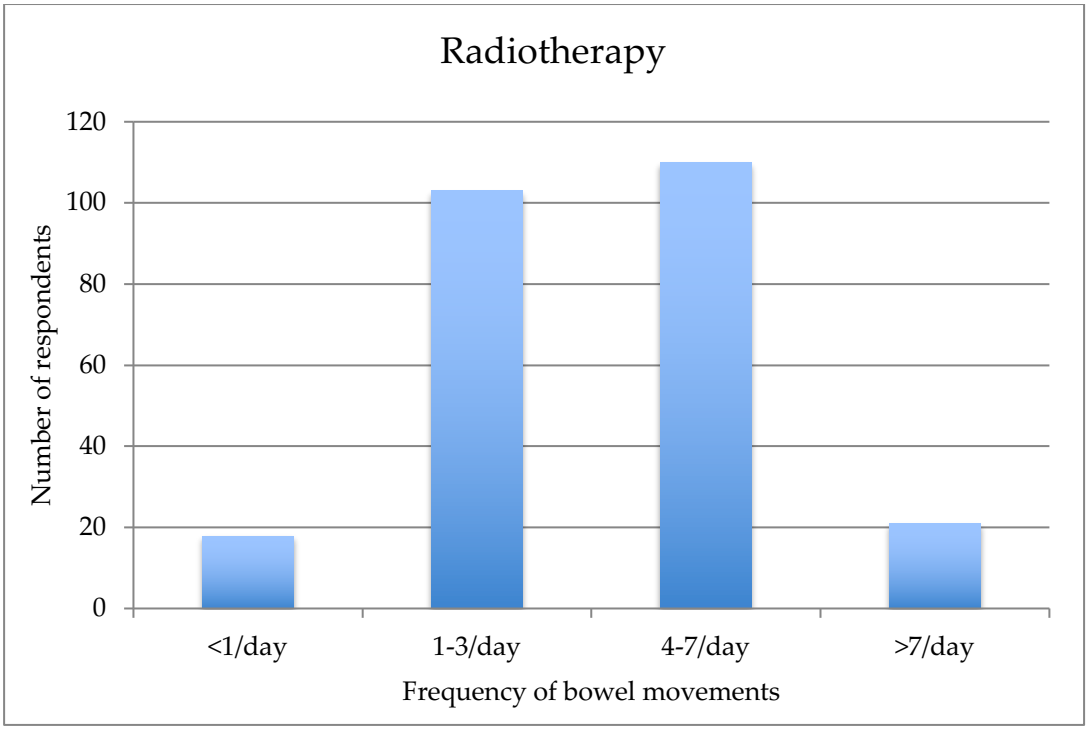
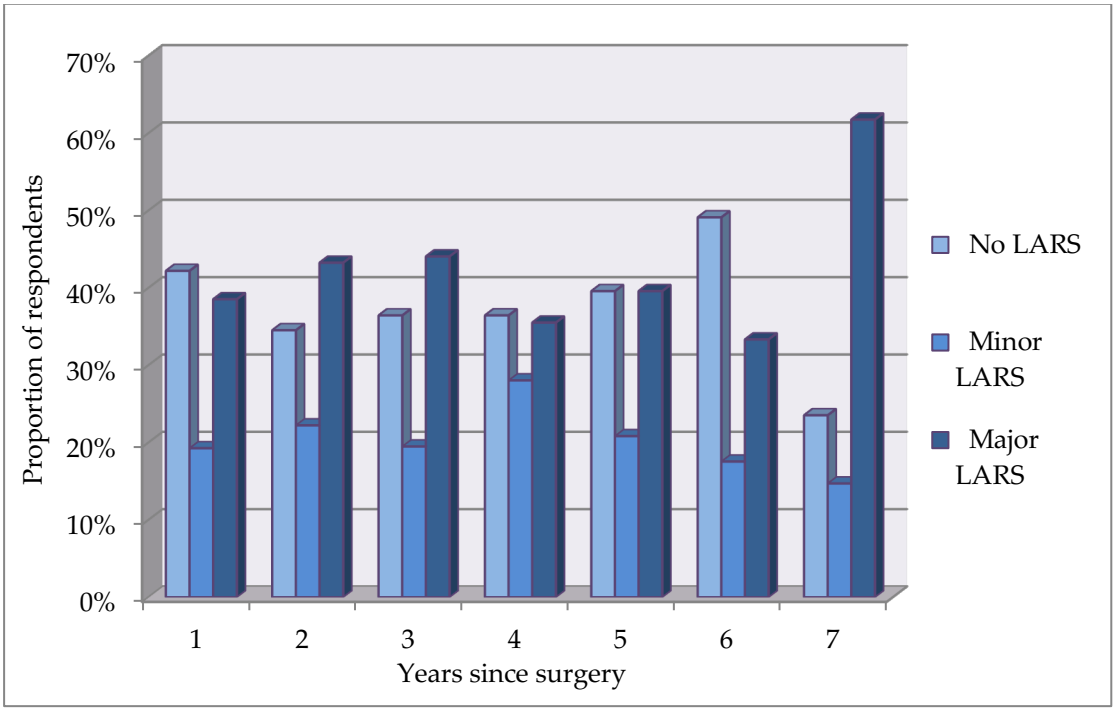


Figure 3.11 LARS category by year since surgery for those in the first seven years since surgery (further years not included as very small numbers in each year). LARS: low anterior resection syndrome.



3.4.9 Quality of life results

Quality of life was significantly different between the three groups with a p-value of <0.0001 on global health, all five functional scales and all nine symptom scales. These results are shown in Tables 3.10 and 3.11 and graphically represented in Figures 3.12 and 3.13. Despite all the scales showing significant differences between groups, the differences in mean scores equate to different clinical relevance[35]. Social functioning showed the greatest difference between groups for the functional scales and diarrhoea was the symptom scale with the greatest difference amongst LARS categories.

Those with no LARS scored significantly better than the general population for global health and all five functional scales, except physical functioning. Respondents with major LARS scored significantly worse than the population for global health and all scales except emotional functioning, see Table 3.12. Those with minor LARS scored significantly better than the population for global health, role functioning and emotional functioning, with no difference in the other scales.

Table 3.13 shows the results for symptom scales and how the population compare with those in the LARS categories. Those with no LARS scored significantly better than the population for fatigue, nausea & vomiting, pain, appetite loss, diarrhoea and financial difficulties, but they scored significantly worse for constipation. The major LARS group scored significantly worse than the population for all symptoms except pain. For the group with minor LARS the results were mixed, with scores significantly better than the population for fatigue and pain, and significantly worse than the population for constipation and diarrhoea; there was no difference for the other symptoms.

Tables 3.14 and 3.15 show the quality of life results for those who underwent radiotherapy and those who did not. Quality of life was not affected by year since operation ($p = 0.088$). Figure 3.14 shows the mean global health for those in the first seven years since surgery (further years were not included as very small numbers in each year).

Table 3.16 gives a breakdown of responses to the individual questions from the QLQ-C30 questionnaire according to LARS category. The results were significantly different across the three LARS categories for all questions ($p = <0.001$ for all, using Goodman and Kruskal's gamma).

Table 3.10 Quality of life results – global health and functional scales (perfect score = 100). p-value shows difference between the three groups using Kruskal-Wallis test. Clinical significance of the difference in mean score between no LARS and major LARS. LARS: low anterior resection syndrome.

	Mean score No LARS	Mean score Minor LARS	Mean score Major LARS	p-value	Score difference no LARS vs. Major LARS	Clinical significance
Global Health	82.9	79.4	69.1	<0.0001	13.8	Moderate
Physical functioning	91.3	89.7	81.5	<0.0001	9.8	Minor
Role functioning	92.6	90.6	75.2	<0.0001	17.5	Moderate
Emotional functioning	90.6	85.6	76.3	<0.0001	14.3	Moderate
Cognitive functioning	89.9	87.2	80.3	<0.0001	9.6	Minor
Social functioning	92.1	85.1	68.2	<0.0001	23.9	Major

Table 3.11 Quality of life results – symptom scales (perfect score = 0). p-value shows difference between the three groups using Kruskal-Wallis test. Clinical significance of the difference in mean score between no LARS and major LARS. LARS: low anterior resection syndrome.

	Mean score No LARS	Mean score Minor LARS	Mean score Major LARS	p-value	Score difference no LARS vs. Major LARS	Clinical significance
Fatigue	14.5	18.5	29.5	<0.0001	15.0	Moderate
Nausea & vomiting	1.5	2.5	7.0	<0.0001	5.5	Minor
Pain	6.1	9.3	19.2	<0.0001	13.1	Moderate
Dyspnoea	9.3	11.3	18.0	<0.0001	8.7	Minor
Insomnia	15.8	22.4	32.7	<0.0001	16.9	Moderate
Appetite loss	3.3	5.2	10.7	<0.0001	7.4	Minor
Constipation	14.9	16.2	25.5	<0.0001	10.6	Moderate
Diarrhoea	3.8	15.4	27.9	<0.0001	24.1	Major
Financial difficulties	4.8	8.5	16.7	<0.0001	11.9	Moderate

Figure 3.12 Quality of life results – global health and functional scales (perfect score = 100) Population normal values are shown[34]. Error bars show the 95% confidence intervals. LARS: low anterior resection syndrome.

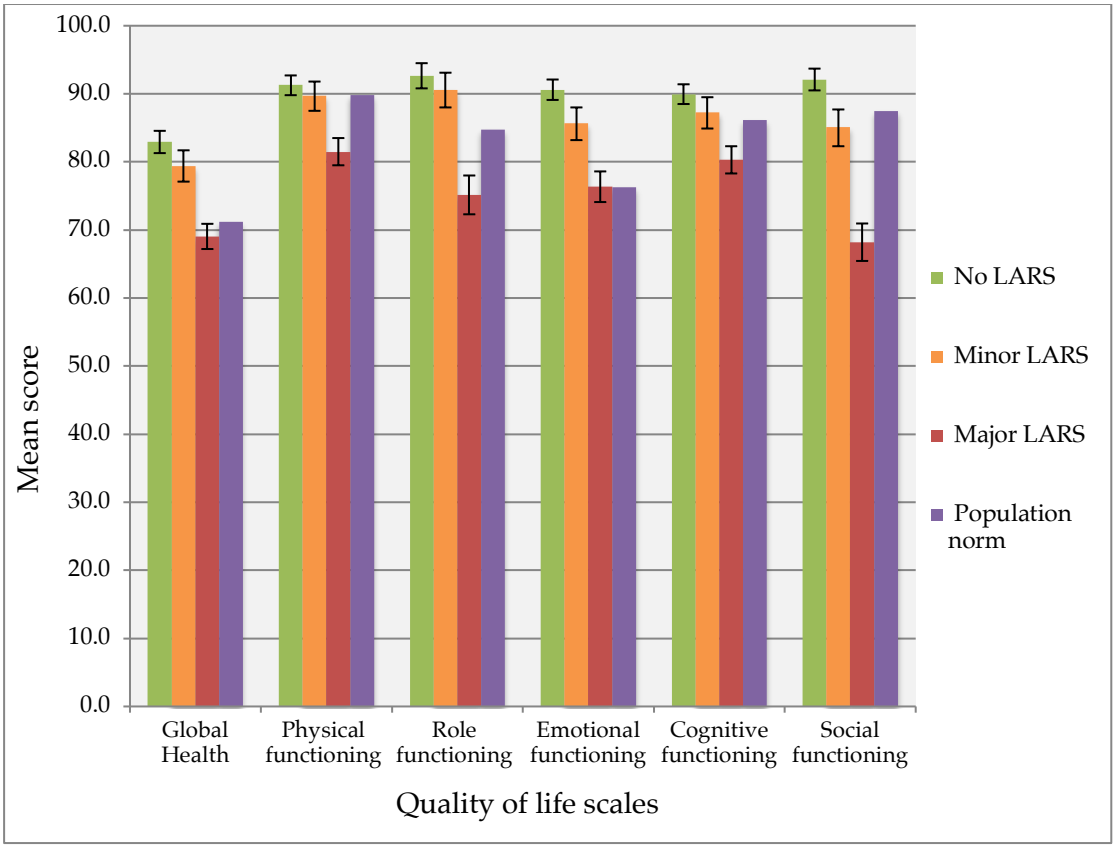


Figure 3.13 Quality of life results – symptom scales (perfect score = 0). Population normal values are shown[34]. Error bars show the 95% confidence intervals. LARS: low anterior resection syndrome.

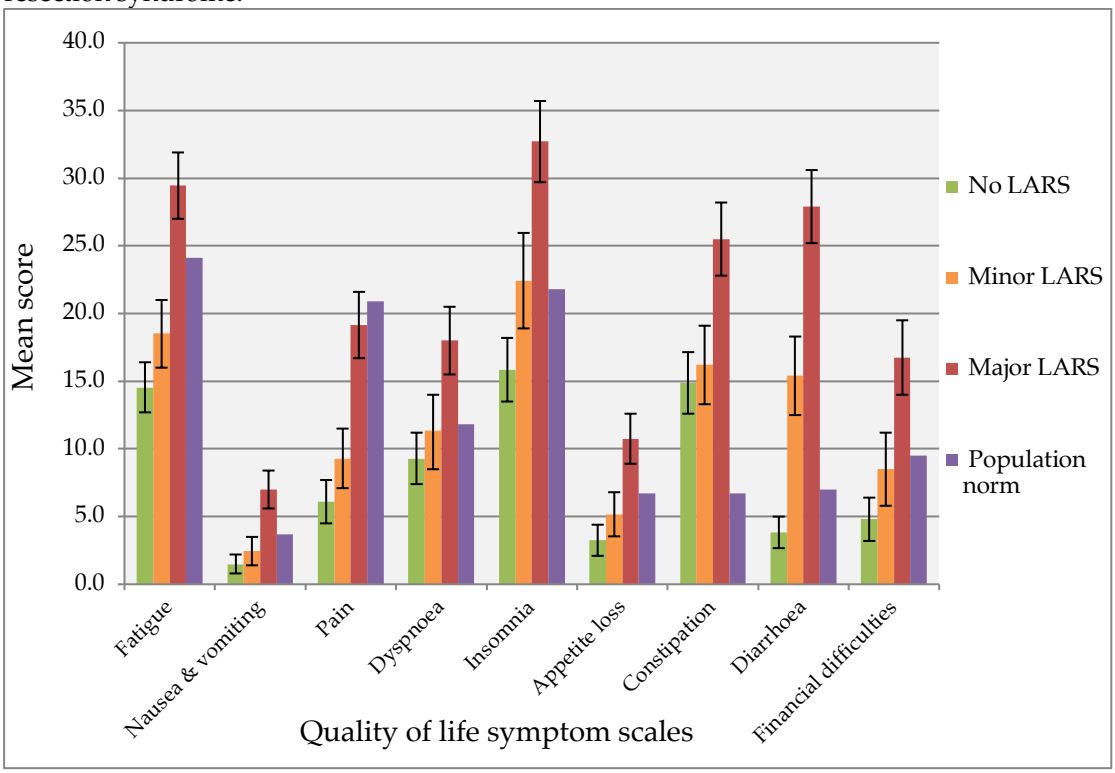


Table 3.12 Quality of life results – mean scores for global health and functional scales (perfect score = 100). Difference between population and LARS groups with one-sample T test. LARS: low anterior resection syndrome.

	Population norm	No LARS	p-value	Minor LARS	p-value	Major LARS	p-value
Global Health	71.2	82.9	<0.0001	79.4	<0.0001	69.1	0.0260
Physical functioning	89.8	91.3	0.0514	89.7	0.8926	81.5	<0.0001
Role functioning	84.7	92.6	<0.0001	90.6	<0.0001	75.2	<0.0001
Emotional functioning	76.3	90.6	<0.0001	85.6	<0.0001	76.3	0.9836
Cognitive functioning	86.1	90.0	<0.0001	87.2	0.3567	80.3	<0.0001
Social functioning	87.5	92.1	0.0010	85.0	0.0723	68.2	<0.0001

Table 3.13 Quality of life results – mean scores for symptom scales (perfect score = 0). Difference between population and LARS groups with one-sample T test. LARS: low anterior resection syndrome.

	Population norm	No LARS	p-value	Minor LARS	p-value	Major LARS	p-value
Fatigue	24.1	14.5	<0.0001	18.5	<0.0001	29.5	<0.0001
Nausea & vomiting	3.7	1.5	<0.0001	2.5	0.0245	7.0	<0.0001
Pain	20.9	6.1	<0.0001	9.3	<0.0001	19.2	0.1710
Dyspnoea	11.8	9.3	0.0101	11.3	0.6932	18.0	<0.0001
Insomnia	21.8	15.8	<0.0001	22.4	0.7354	32.7	<0.0001
Appetite loss	6.7	3.3	<0.0001	5.2	0.0698	10.7	<0.0001
Constipation	6.7	14.9	<0.0001	16.2	<0.0001	25.5	<0.0001
Diarrhoea	7.0	3.8	<0.0001	15.4	<0.0001	27.9	<0.0001
Financial difficulties	9.5	4.8	<0.0001	8.5	0.4575	16.7	<0.0001

Table 3.14 Quality of life results - global health and functional scales (perfect score = 100). QOL with and without radiotherapy. CI: confidence intervals; NA: neoadjuvant.

	Mean score No NA radiotherapy (95% CI)	Mean score NA radiotherapy (95% CI)	p-value
Global Health	76.9 (75.6 – 78.2)	75.1 (72.6 – 77.6)	0.241
Physical functioning	87.7 (86.5 – 88.9)	84.3 (81.5 – 87.0)	0.237
Role functioning	86.8 (85.1 – 88.4)	79.2 (75.4 – 82.9)	<0.001
Emotional functioning	84.0 (82.6 – 85.4)	82.3 (79.5 – 85.1)	0.508
Cognitive functioning	85.6 (84.3 – 86.9)	85.1 (82.6 – 87.5)	0.990
Social functioning	83.2 (81.5 – 84.8)	72.9 (69.2 – 76.6)	<0.001

Table 3.15 Quality of life results – symptom scales (perfect score = 0). QOL with and without radiotherapy. CI: confidence intervals; NA: neoadjuvant.

	Mean score No NA radiotherapy (95% CI)	Mean score NA radiotherapy (95% CI)	p-value
Fatigue	21.2 (19.7 – 22.8)	22.4 (19.3 – 25.6)	0.942
Nausea & vomiting	3.7 (3.0 – 4.5)	4.8 (3.1 – 6.5)	0.299
Pain	11.6 (10.1 – 13.0	14.2 (11.2 – 17.2)	0.117
Dyspnoea	13.1 (11.5 – 14.7)	13.9 (10.8 – 17.1)	0.898
Insomnia	24.0 (22.0 – 26.0)	25.3 (21.6 – 28.9)	0.510
Appetite loss	6.5 (5.4 – 7.6)	7.4 (5.4 – 9.5)	0.306
Constipation	19.8 (18.0 – 21.6)	18.6 (15.3 – 21.8)	0.470
Diarrhoea	14.7 (13.1 – 16.3)	20.8 (17.4 – 24.2)	<0.001
Financial difficulties	9.6 (8.0 – 11.2)	13.6 (10.3 – 16.9)	0.002

Figure 3.14 Mean global health by year post-operation for those in the first seven years since surgery (further years not included as very small numbers in each year). Error bars show the 95% confidence intervals.

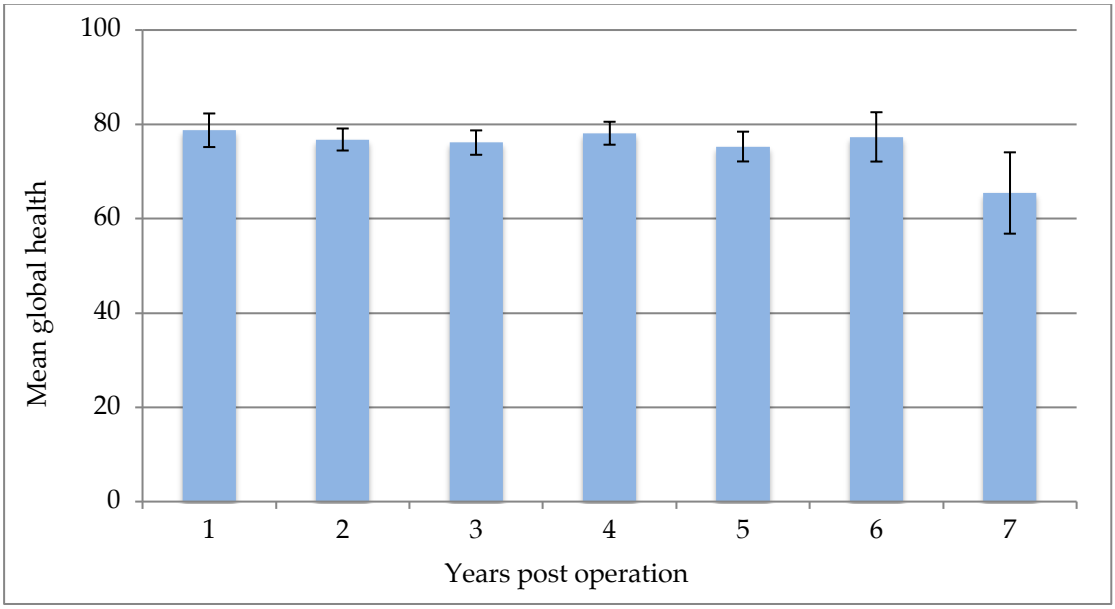


Table 3.16 Responses to questions on the QLQ-C30 questionnaire by LARS category.

		Not at All		A Little		Quite a Bit		Very Much		p-value
		N	%	N	%	N	%	N	%	
Q1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or suitcase?	No LARS	266	65.4	108	26.5	21	5.2	12	2.9	<0.001
	Minor LARS	148	62.4	57	24.1	22	9.3	10	4.2	
	Major LARS	204	45.9	129	29.1	61	13.7	50	11.3	
Q2. Do you have any trouble taking a long walk?	No LARS	279	68.6	75	18.4	28	6.9	25	6.1	<0.001
	Minor LARS	150	63.6	45	19.1	25	10.6	16	6.8	
	Major LARS	191	43.1	109	24.6	72	16.3	71	16.0	
Q3. Do you have any trouble taking a short walk outside of the house?	No LARS	362	90.0	26	6.5	9	2.2	5	1.2	<0.001
	Minor LARS	205	87.6	23	9.8	4	1.7	2	0.9	
	Major LARS	331	74.5	74	16.7	23	5.2	16	3.6	
Q4. Do you need to stay in bed or a chair during the day?	No LARS	362	88.7	33	8.1	12	2.9	1	0.2	<0.001
	Minor LARS	210	88.6	19	8.0	6	2.5	2	0.8	
	Major LARS	341	76.5	73	16.4	26	5.8	6	1.3	
Q5. Do you need help with eating, dressing, washing yourself or using the toilet?	No LARS	397	97.8	4	1.0	2	0.5	3	0.7	<0.001
	Minor LARS	228	95.8	5	2.1	3	1.3	2	0.8	
	Major LARS	410	91.7	20	4.5	14	3.1	3	0.7	
Q6. Were you limited in doing either your work or other daily activities?	No LARS	346	84.8	46	11.3	8	2.0	8	2.0	<0.001
	Minor LARS	192	81.4	33	14.0	6	2.5	5	2.1	
	Major LARS	248	55.7	118	26.5	44	9.9	35	7.9	
Q7. Were you limited in pursuing your hobbies or other leisure time activities?	No LARS	343	84.5	45	11.1	8	2.0	10	2.5	<0.001
	Minor LARS	182	77.4	42	17.9	4	1.7	7	3.0	
	Major LARS	232	52.1	120	27.0	49	11.0	44	9.9	
Q8. Were you short of breath?	No LARS	317	77.7	73	17.9	13	3.2	5	1.2	<0.001
	Minor LARS	174	73.4	51	21.5	7	3.0	5	2.1	
	Major LARS	281	63.0	106	23.8	42	9.4	17	3.8	
Q9. Have you had pain?	No LARS	339	83.9	45	11.1	14	3.5	6	1.5	<0.001
	Minor LARS	165	69.3	60	25.2	10	4.2	3	1.3	
	Major LARS	255	57.3	119	26.7	48	10.8	23	5.2	
Q10. Did you need to rest?	No LARS	260	63.9	122	30.0	17	4.2	8	2.0	<0.001
	Minor LARS	141	59.7	74	31.4	18	7.6	3	1.3	
	Major LARS	186	42.2	172	39.0	56	12.7	27	6.1	
Q11. Have you had trouble sleeping?	No LARS	260	63.7	112	27.5	26	6.4	10	2.5	<0.001
	Minor LARS	126	52.9	72	30.3	32	13.4	8	3.4	
	Major LARS	169	38.1	154	34.7	81	18.2	40	9.0	
Q12. Have you felt weak?	No LARS	296	72.7	89	21.9	18	4.4	4	1.0	<0.001
	Minor LARS	149	63.1	71	30.1	12	5.1	4	1.7	
	Major LARS	205	46.0	162	36.3	55	12.3	24	5.4	
Q13. Have you lacked appetite?	No LARS	375	91.9	27	6.6	5	1.2	1	0.2	<0.001
	Minor LARS	203	85.3	33	13.9	2	0.8	0	0	
	Major LARS	328	73.4	100	22.4	13	2.9	6	1.3	
Q14. Have you felt nauseated?	No LARS	385	94.4	19	4.7	3	0.7	1	0.2	<0.001
	Minor LARS	215	90.3	20	8.4	2	0.8	1	0.4	
	Major LARS	338	75.8	85	19.1	17	3.8	6	1.3	

		Not at All		A Little		Quite a Bit		Very Much		p-value
		N	%	N	%	N	%	N	%	
Q15. Have you vomited?	No LARS	402	98.5	4	1.0	2	0.5	0	0	<0.001
	Minor LARS	230	97.0	6	2.5	1	0.4	0	0	
	Major LARS	407	91.1	31	6.9	7	1.6	2	0.4	
Q16. Have you been constipated?	No LARS	268	65.7	106	26.0	26	6.4	8	2.0	<0.001
	Minor LARS	146	61.6	68	28.7	22	9.3	1	0.4	
	Major LARS	213	47.8	149	33.4	60	13.5	24	5.4	
Q17. Have you had diarrhea?	No LARS	365	89.7	38	9.3	3	0.7	1	0.2	<0.001
	Minor LARS	150	63.0	69	29.0	16	6.7	3	1.3	
	Major LARS	185	41.8	168	37.9	67	15.1	23	5.2	
Q18. Were you tired?	No LARS	226	55.9	148	36.6	24	5.9	6	1.5	<0.001
	Minor LARS	96	40.7	115	48.7	22	9.3	3	1.3	
	Major LARS	129	29.1	202	45.6	77	17.4	35	7.9	
Q19. Did pain interfere with your daily activities?	No LARS	366	90.1	28	6.9	7	1.7	5	1.2	<0.001
	Minor LARS	201	84.5	32	13.4	3	1.3	2	0.8	
	Major LARS	295	66.7	93	21.0	31	7.0	23	5.2	
Q20. Have you had difficulty in concentrating on things like reading a newspaper or watching television?	No LARS	367	90.0	33	8.1	7	1.7	1	0.2	<0.001
	Minor LARS	199	84.3	28	11.9	7	3.0	2	0.8	
	Major LARS	313	70.3	98	22.0	24	5.4	10	2.2	
Q21. Did you feel tense?	No LARS	314	77.0	82	20.1	11	2.7	1	0.2	<0.001
	Minor LARS	161	67.6	64	26.9	11	4.6	2	0.8	
	Major LARS	214	48.4	158	35.7	58	13.1	12	2.7	
Q22. Did you worry?	No LARS	284	69.6	98	24.0	24	5.9	2	0.5	<0.001
	Minor LARS	138	58.0	78	32.8	17	7.1	5	2.1	
	Major LARS	198	44.6	172	38.7	52	11.7	22	5.0	
Q23. Did you feel irritable?	No LARS	315	77.6	79	19.5	10	2.5	2	0.5	<0.001
	Minor LARS	152	64.7	72	30.6	8	3.4	3	1.3	
	Major LARS	213	47.8	156	35.0	61	13.7	16	3.6	
Q24. Did you feel depressed?	No LARS	331	81.3	61	15.0	12	2.9	3	0.7	<0.001
	Minor LARS	171	72.2	46	19.4	13	5.5	7	3.0	
	Major LARS	240	54.1	146	32.9	37	8.3	21	4.7	
Q25. Have you had difficulty remembering things?	No LARS	239	58.7	143	35.1	22	5.4	3	0.7	<0.001
	Minor LARS	129	55.1	86	36.8	13	5.6	6	2.6	
	Major LARS	177	40.1	202	45.8	43	9.8	19	4.3	
Q26. Has your physical condition or medical treatment interfered with your family life?	No LARS	336	82.6	56	13.8	9	2.2	6	1.5	<0.001
	Minor LARS	1632	69.1	53	22.5	17	7.2	3	1.3	
	Major LARS	04	46.0	138	31.2	66	14.9	35	7.9	
Q27. Has your physical condition or medical treatment interfered with your social activities?	No LARS	329	80.8	58	14.3	17	4.2	3	0.7	<0.001
	Minor LARS	147	62.0	68	28.7	18	7.6	4	1.7	
	Major LARS	152	34.2	162	36.4	82	18.4	49	11.0	
Q28. Has your physical condition or medical treatment caused you financial difficulties?	No LARS	368	90.4	24	5.9	10	2.5	5	1.2	<0.001
	Minor LARS	195	82.6	28	11.9	7	3.0	6	2.5	
	Major LARS	305	68.4	90	20.2	19	4.3	32	7.2	

3.4.10 Comments from participants

Along with returning their questionnaire in the post, 23 patients also sent either a note or a letter along with it. These responses were uninvited but detail the experience of those living with symptoms of LARS. A small selection of these responses is included below.

"Difficult to answer some questions as I compare things like quality of life with the worst misery and zilch quality of life, and my friends and peers who seem to 'have a life'. I try to measure quality in the things like being able to get to the toilet by myself, being actually able to get out for a coffee but I really want to be able to go on a holiday or be able to work at a fulfilling or even a decent basic job and stay/visit with friends – none of which I can do now."
55F, LARS score = 41 (major LARS)

"The first few weeks after the reversal were an absolute nightmare but this fortunately soon changed and my bowel settled (if that's the right word) to a pattern of usually producing nothing for two days and then requiring me to make a number of visits to the toilet on the third day over a period of about three hours, the first visit I usually get a warning of about two or three minutes, subsequent visits can involve a more athletic dash (may become a problem with increasing age) after this we settle back to the routine. The problem to my work, and social life is never being quite sure of the timing and sometimes I can need to go to the toilet on consecutive days and although this is unusual it is a frequent worry to my wife and I when travelling or when I am attending one of my many meetings, hence my response to the questions about worry, stress and depression."
66M, LARS score = 42 (major LARS)

"Following reversal my life has changed, I am ruled by my bowels, have to be careful about what I eat and drink. Life will never be the same. But what choice did I have? I had to go with the treatment good that it was. I am still alive for my family."
63M, LARS score = 32 (major LARS)

"Given that my cancer is cured, I accept LARS as the price to pay."
79M, LARS score = 41 (major LARS)

"You do not ask what changes there have been to the timing, frequency and duration of the 'opening of my bowels', which are the biggest and most affecting changes to my life. Before I went once, first thing in the morning and it took about one minute. Now, my 'active' time is between 0100hrs and 0700hrs, around three times and each one can last half an hour, which interferes with my sleep."
72M, LARS score = 30 (major LARS)

"What bothers me most is that I can never predict what a day will bring."
70F, LARS score = 37 (major LARS)

"I have to go to the toilet about 6 times a day. I pass about 2 pieces each time. I never feel as if I have finished, but cannot pass anymore by staying there. Before I go to the toilet it becomes uncomfortable and I must go, where ever I am. I can hold on but becomes more uncomfortable the longer I leave it. I have not had any accidents. When I go it is normally on the medium to firm side. It is thought that my bowel does not expand as it should, to collect more before going to the toilet. I have had far more flatus – wind, than before the operation. I have to pass the wind as it makes it very uncomfortable, near to pain, in the area of the operation. I cannot control this all the time. These are the main problems from the operation. I am in good health and try not to get me down, or stop me from doing anything I would normally do. It is what it is and I try to get on with my life."
62M, LARS score = 36 (major LARS)

3.5 Discussion

This study assessed the prevalence of LARS in the UK population for the first time following the UK validation of the LARS score. LARS was found to affect a considerable proportion of patients following anterior resection, with 41% experiencing major LARS and 63% experiencing LARS in some form. The majority of patients who have undergone an anterior resection will have to live with bowel dysfunction affecting their quality of life. These results are similar to those from the UK validation of the LARS questionnaire, which found that 47% of patients had major LARS and a further 23% had minor LARS[28]. The results are also very similar to a study of LARS prevalence in Danish patients, which showed 41% major LARS and 23% minor LARS[11]. Prior to the development of the LARS score, the lack of measurement tool meant that studies were limited to assessment of individual

symptoms such as urgency or incontinence, making estimation of the proportion of patients who had LARS very difficult[21]. Previous published reviews about LARS quote wide ranging estimates of the proportion affected including: 10-70%[36] up to 60%[21], up to 80%[37] and up to 90%[7]. This may partly explain why the proportion affected has not been appreciated; one study of Spanish and American surgeons found that they have good theoretical knowledge about LARS but underestimate the probability of patients suffering from it[38].

Clustering was the most frequently experienced symptom in these respondents followed by flatus incontinence and urgency. Even the least frequently experienced symptom, faecal incontinence, was still experienced by nearly half of respondents. A meta-analysis of functional symptoms after anterior resection calculated pooled incidence of symptoms and also found clustering to be the most frequently experienced, with pooled incidence of 59%[21]. The results for pooled proportions of symptoms from the meta-analysis are lower than the current study for all of the symptoms involved[21].

Clustering can be defined as "numerous bowel movements occurring within a short time period"[39], essentially reflecting a sensation of incomplete emptying that requires a return to the toilet multiple times[40]. The term 'stool fragmentation' is also used to describe the same problem[39]. It is a symptom that is frequently assessed in studies of bowel function[37] and one that patients find troublesome as it affects their daily activities and quality of life[9].

LARS was associated with increased stool frequency; the majority of those with major LARS had a frequency of 4-7 times per day. Interestingly, for those with major LARS the proportion opening their bowels less than once daily (7.6%) was lower than this proportion in those without LARS (11.5%) and in all respondents (8.8%). Despite this result, the proportion reporting constipation on the QLQ-C30 questionnaire was significantly higher for those with LARS. It is possible that what these patients are reporting as 'constipation' is actually difficulty with rectal evacuation or incomplete emptying, both of which are known to be symptoms of LARS[7].

The risk factors for LARS identified by this study are neoadjuvant radiotherapy, defunctioning stoma, female gender and younger age (≤65); these had all been identified as risk factors for LARS by previous studies[11,41,42]. Neoadjuvant

radiotherapy is an established risk factor for bowel dysfunction following anterior resection[41,43]. Radiotherapy induces physiological changes including reduced squeeze pressures, capacity and rectal distensibility[44] with scarring of the anal sphincters, possibly due to fibrosis[45,46]. Defunctioning stoma has also been previously identified as a risk factor for LARS[42]. It is likely that in this study it also acts as a surrogate marker for anastomotic height. With the methodology used it was not possible to collect this information and confirm this. However, defunctioning stoma has been shown to increase risk independently of tumour height[42], this may be partly due to the effects of diversion colitis on the neorectum[47] although the exact mechanism is not fully understood.

Studies have shown conflicting results with regard to the effect of gender on functional outcomes following anterior resection[11,48]. It is accepted that bowel function in women is potentially made worse by the impact of obstetric factors on pelvic floor function but to fully determine whether this is the only factor involved would require further studies. Three previous studies using the LARS questionnaire have identified younger age as a risk factor for LARS[11,13,42] despite baseline bowel dysfunction being more prevalent in older people[49]. One possible explanation for this finding is that younger patients have better pre-operative function and therefore a greater perceived difference between their function before and after anterior resection[11]. Younger people are more likely to be planning a return to employment and the symptoms of LARS can make this very difficult, as mentioned in the comments received from respondents. Another possible reason which has been suggested for this result, is that those older patients with pre-existing poor function may have had an APER or anterior resection with end colostomy rather anastomosis, skewing the results[42]. This would again, require prospective studies, to fully understand the contributing factors for this result.

This study did not identify any relationship between the time since surgery and risk of LARS. Quality of life was also not dependent on number of years since operation. These findings are in keeping with a follow-up study from the Dutch TME trial, which showed that even 14 years later, many patients remained symptomatic from LARS[13]. This study excluded patients in the first twelve months post surgery who have been previously shown to have worse function than patients at twelve months[8]. These findings have implications for the discussions about function held with patients in the first year during surgery and beyond this point.

The results from this study confirm that LARS has a detrimental effect on all aspects of quality of life, with the greatest effect on social functioning, findings that are in keeping with those from previous studies[8,12,17]. The individual symptoms that contribute to LARS have been shown to individually affect QOL so it is no surprise that a combination of these problems has an even greater effect. On the individual symptom scales, diarrhoea showed the greatest difference between those with and without LARS but financial difficulties, insomnia, pain and fatigue were the next most clinically relevant scores, showing that LARS affects a number of aspects of QOL. Even symptoms seemingly unrelated to LARS such as shortness of breath, nausea, weakness and poor appetite were all significantly more frequent in those with LARS. Some of the emotional and mental aspects of LARS including feeling worried, depressed, tense and irritable, along with difficulties concentrating and with memory may be partially the result of problems with sleep but, as one respondent mentioned in their comment, these can also relate directly to bowel symptoms.

A Cochrane review comparing QOL in patients who have undergone anterior resection and APER showed no difference and suggested that LARS accounts for the lack of benefit from sphincter preserving surgery[50]. Cancer survivors have previously been shown to have worse QOL than the population[51]. Interestingly in this study, those patients who had undergone anterior resection and did not have LARS actually had better QOL than the background population. This could be explained by the finding that having cancer can lead to positive adaptations including improved life outlook and enhanced relationships[52].

Just over half of the respondents underwent a defunctioning stoma at the time of surgery, this compares with 57% in the Dutch TME trial[53]. The UK National Bowel Cancer Audit (NBCA) 2018 report shows that between 2013-2016, 77% of anterior resections were covered by a stoma[29]. The proportion of respondents in the current study who had a stoma, would be expected to be lower than national data as the participants are survivors with good outcomes (see below). The risk factors for stoma formation were male gender, open/converted surgery and neoadjuvant radiotherapy. These are all known to be risk factors for anastomotic leak[54,55] and therefore inherently would be potential reasons for stoma formation. Male gender has been shown to be a risk for conversion from laparoscopic surgery to open and the narrower male pelvis can make rectal cancer surgery technically challenging[56]. Preoperative radiotherapy has been previously shown to increase the likelihood of

stoma formation although the same study showed no difference rates of stoma between genders[57]. It is not surprising that adjuvant chemotherapy led to delays with closure of stoma, this has been identified by several studies[58-60] and is well recognised in clinical practice.

In this study, 7.2% underwent neoadjuvant SCRT and 16% LCRT, with increased use in younger patients. This compares with national audit data showing 8% of patients had SCRT and 26% LCRT; the national audit also found that younger patients were more likely to undergo radiotherapy[29].

This questionnaire identified highly variable results for rates of defunctioning stoma, use of neoadjuvant therapy including short course and long course radiotherapy, use of adjuvant therapy, mode of surgery and conversion rates from laparoscopic to open surgery across the PICs that took part in the study. This finding reflects the complexities involved in the management of rectal cancer treatment but also raises several questions about why this variation exists. The use of a defunctioning stoma for anterior resection ranged from 10-100% between populations that would be expected to be fairly similar in their pathology. The NBCA also reported marked variation in rates of stoma use, neoadjuvant therapy, laparoscopic surgery and adjuvant chemotherapy[29]. One of the recommendations of the audit report is a better understanding of regional differences in treatment, and the reasons underlying this[29]. The level of variation in practice confirms that complex factors are involved in decision-making when treating patients with rectal cancer, and further studies to look directly at this would potentially be very useful.

The LARS questionnaire is easily completed and ideally suited to routine use during follow-up either in outpatients or via telephone follow-up. It is also ideal as a research tool to permit comparison of outcomes with a consistent outcome measure. The questionnaire has been designed to be used as a universal international measure for measuring LARS[27] and has already been translated and validated in several languages. Although the questionnaire classifies patients into those with major LARS, minor LARS and no LARS, in some regards this is an artificial dichotomy as in reality the symptoms are on a spectrum, for example someone scoring 19 and 20 will probably have very similar experiences. There are also many other symptoms, which affect patients and impact on QOL, which are not included in the questionnaire, including: urinary incontinence, subfertility, erectile dysfunction, neuropathy from chemotherapy, dyspareunia and premature menopause[10,61,62].

It has previously been shown that surgeons and oncologists do not have a thorough understanding of which symptoms are most troublesome to patients[63]. The results from this study indicate a need for careful counselling of patients and discussion of this potential side effect through the process of joint decision-making. This can be very challenging at the time of cancer diagnosis, particularly in rectal cancer, as the treatment options can be complex. Increased use of neoadjuvant therapy has improved survival from rectal cancer but this treatment has a long-term impact on function and needs to be reserved for those who will benefit from it. There is a need to understand more about how to predict response in individual patients and work in ongoing, with an online tool to predict bowel dysfunction now available[42].

The demographics of the respondents to the questionnaire reflect those of the rectal cancer population. Rectal cancer is more common in men, who make up over 60% of those with rectal in the UK[1]. The respondents to the questionnaire are those with good outcomes since they have survived beyond twelve months and are not receiving ongoing chemotherapy or have local recurrence; this means that the results are only applicable to this group. The proportions of all rectal cancer patients undergoing defunctioning stoma, neoadjuvant and adjuvant therapy would therefore be expected to be higher than those shown in the results.

The response rate to the questionnaire, 53%, is reasonable given that this was an uninvited questionnaire which came directly from the study team (and not the patient's usual surgical department) and that the participants were an average of three years post surgery, meaning that some would have moved address. A wide range of NHS Trusts were included across England and Scotland, including both teaching hospitals in cities and district general hospitals in both urban and rural areas.

Limitations of the study include that the baseline function / QOL in these patients is unclear. Due to the retrospective approach and the methodology used it was not possible to guarantee that all potentially eligible participants were included or to characterise the non-respondents. In surveys of cancer-related experience, non-respondents are more likely to be young, non-White and socioeconomically deprived[64]. The sample size was based on feasibility and no power calculation was carried out prior to the study. This was partly due to the lack of evidence about the expected proportions of patients with LARS at the time the study was designed. It is possible that patients may have been inaccurate in their knowledge of their

treatment, although this does not seem to be the case as most items were completed and many respondents added extra information. It was not possible to collect data about some variables including tumour stage, tumour height and anastomotic configuration. These variables are often inconsistently recorded and heterogeneous in nature[21] and this information would be best captured with a prospective approach. In hindsight, it would have been interesting to ask participants questions about the management of their symptoms, whether they have received support with them and how they are coping with their symptoms. These questions would have provided additional relevant information but would also have lengthened the questionnaire and potentially caused concern or anxiety for participants.

A prospective multi-centre study would not only help to fully understand the risk factors and causes of LARS but could also cover a range of other related questions including reasons for stoma formation and non-closure, risks for anastomotic leak, the effect of post-operative morbidity on long term QOL and comparison in QOL with patients having APER. It would be possible to take into account baseline function, obstetric injuries, co-morbidities and presenting symptoms, all factors which may well play a part in the development of LARS.

The letters and comments received from participants were unexpected but they are evidence of the clear need that people with LARS have to talk about the problems they are experiencing. Several participants also phoned to discuss their problems in more detail, often mentioning that they found it difficult to talk to family or friends due to embarrassment. A large number expressed a wish to receive a lay copy of the results of this study (which was offered in the participant information sheet). The emphasis for these people is on the effect that symptoms have on their daily life and ability to carry out activities that other people take for granted, for example going to work, going on holiday or visiting friends. These issues have been identified by qualitative studies exploring the problems experienced by those with LARS[9,65]. Respondents acknowledged that they have survived cancer and they are grateful for this but they surely deserve better recognition and treatment for LARS, a long term condition, which is a side effect of treatment and affects so many of them.

Routine use of the LARS questionnaire during follow-up would allow identification of patients who might benefit from treatment and referral of these patients. It is only through routine assessment, intervention and then structured analysis of intervention outcomes that treatment for LARS will be improved[10]. Given the scale of

the problem, more work is needed on treatment strategies including to determine the optimal time for treatment and who should oversee this. There is currently no specific treatment for LARS and a lack of ownership of these patients outside of specialist centres with an interest in functional problems[7,66]. Colorectal nurse specialists, who are primarily responsible for follow-up of rectal cancer patients, are usually the first to identify that patients are experiencing symptoms. They may be able to provide advice and support depending on their level of experience but management is usually symptom based[7].

Initial treatment measures for LARS may include dietary advice, laxatives, bulking agents, loperamide and suppositories[67]. Patients who do not improve with these measures may be referred to specialist pelvic floor clinics or tertiary units who specialise in these problems but this often requires travelling, which can clearly be difficult for those with LARS. Further options for therapy may include bowel irrigation, biofeedback or sacral nerve stimulation[7,67]. A systematic review assessing the use of pelvic floor rehabilitation, including biofeedback, following anterior resection, showed that these therapies can improve functional outcome[68]. However, the studies included were either case control or cohort studies, and there is a lack of trials in this area. In addition to physical treatment, these patients are also likely to need psychological support[69]. There have been recent calls for a "holistic, multidisciplinary and systematic approach" to treatment for these patients[70] but surely the first step towards this would be introducing assessment for LARS into routine practice when following up patients after anterior resection.

3.6 Conclusions

LARS affects a considerable proportion of patients in the UK following anterior resection, it is detrimental to quality of life and does not resolve with time. Information about LARS needs to be included in patient counselling and consent prior to treatments for rectal cancer.

The LARS score should be routinely used in the clinical follow up of all rectal cancer patients who have undergone anterior resection, to systematically identify those who would benefit from treatment. Rectal cancer patients are usually followed up either by their surgical team or a specialist nurse. The LARS questionnaire should be used at each patient encounter as a screening tool, completed prior to the appointment if

time is limited. This would identify patients who need a more detailed exploration of their symptoms and/or any management options, including onward referral for specialist management or support groups as appropriate. Focused research studies are needed to determine who is best placed to manage LARS – surgeons, specialist nurses or specialist gastroenterologists, and whether this management should this be provided locally or as a tertiary service in pelvic floor units so that relevant investigations and therapeutic options such as biofeedback can be utilised.

This study has confirmed the need for prospective studies to truly understand the risk factors for LARS, as there are some variables, for example, baseline function, which can only be reliably captured in this way. The prospective approach will be the only way to explore the reasons why LARS arises in individual patients. A study of this type would also be an ideal opportunity to explore the reasons behind variations in decision-making and clinical practice during the treatment of rectal cancer.

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Chapter 4. Diffusion-weighted MRI for the prediction and assessment of response to neoadjuvant chemoradiotherapy in rectal cancer

4.1 Introduction

Neoadjuvant long-course chemoradiotherapy (LCRT) is used in locally advanced rectal cancer to decrease the risk of local recurrence and to facilitate oncologically complete resection. In tumours that respond to treatment it leads to downstaging and downsizing and can even lead to a complete response (CR), with disappearance of the tumour in 11-39% of patients[1]. Complete response to LCRT is a good prognostic indicator with reduced recurrence and improved disease-free survival[2]. A proportion of tumours, however, do not respond to LCRT and continue to progress during treatment. Although neoadjuvant LCRT has clear benefits for some patients, it is also associated with short-term toxicity and long-term effects on function[3]. As shown in chapter 3, LCRT increases the risk of patients developing low anterior resection syndrome (LARS), which has a negative impact on their quality of life. The physiological changes underlying LARS are individual and the exact factors leading to variations in functional outcome are not yet clear.

Prediction of response to neoadjuvant LCRT, from the time of initial staging, would allow therapy to be tailored to individual patients, according to whether or not they would benefit from it. This could also allow intensification of treatment with a radiotherapy boost or a change of chemotherapy agent, or in patients for whom a poor response is predicted, the avoidance of LCRT altogether with expedited surgery. This, more rational use of LCRT, would potentially improve functional outcomes for those patients who avoid unnecessary LCRT, one of the major risk factors for a poor functional outcome[4].

At re-staging following LCRT, accurate identification of CR would also be useful, allowing selection of an alternative operative approach such as local excision, which facilitates preservation of sphincters. In patients with a complete response, a 'watch and wait' policy has been advocated by some, with intensive surveillance and avoidance of surgery altogether for a proportion of patients[5]. Identification of a tool that could predict response would, therefore, be very useful for clinical practice. Several imaging and molecular targets, with potential as biomarkers, have been investigated.

T2-weighted magnetic resonance imaging (MRI) scans are widely and routinely used for the diagnosis and initial staging of rectal cancer and have been shown to have high sensitivity and specificity for T staging and assessment of circumferential margin (CRM) involvement[6]. However, a study assessing the use of MRI for restaging following LCRT showed poor overall accuracy for assessing T-stage (52%); identification of CR was also challenging due to difficulty in differentiating between areas of residual viable tumour and areas of fibrosis[7]. The assessment of response to LCRT using MRI has been formalised into a grading system, mrTRG (tumour regression grade). This imaging marker has been shown to predict disease free and overall survival and therefore patient prognosis[8], it has recently become an established and widely used method for the reassessment of tumours following LCRT. Since mrTRG is measured on post treatment scans, it does not, however, have any capacity for the prediction of response.

Diffusion-weighted MRI (DWI) is a functional imaging technique, which utilises the way in which different tissues affect the dynamics of water molecule diffusion, to provide detailed information about tumours and peri-tumoral changes[9].

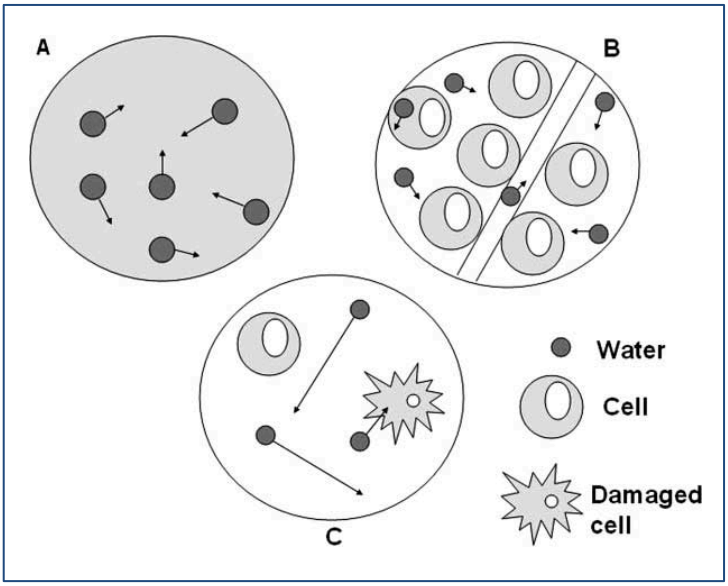
Water molecules are naturally in constant random (Brownian) motion; this permits free diffusion where molecules migrate down a concentration gradient[10,11]. Inside the body, within tissues, this motion is affected and restricted by tissue cellularity, molecules and cell membranes acting as a barrier to movement[10], see Figure 4.1. The motion of water molecules can therefore provide information about tissue cellularity and whether cell membranes are intact[10], factors that can be affected by neoplastic processes including cell lysis, oedema, scarring, fibrosis, inflammation or infiltration[12,13].

DWI assesses the movement of water molecules by 'labelling' these molecules in a volume of tissue and then resampling the same tissue at time intervals to determine the proportion of labelled molecules that remain present[13]. This is done by using radiofrequency to alter the physical properties of protons, in a similar way to standard MRI[13]. This measurement of the movement and restriction of water molecules is then quantified as the 'apparent diffusion coefficient' (ADC)[10].

Measurement of the ADC can facilitate assessment of molecular and metabolic changes at a cellular level, before morphological and structural changes even occur and therefore before these changes become visible[11,14]. Assessment of ADC and the

signal intensity on DWI can allow differentiation between residual tumour and areas of altered tissue cellularity for other reasons including fibrosis, necrosis or inflammation[15] and allows "inferences to be made about the microstructure of the cellular environment"[16].

Figure 4.1 Schematic illustrating movement of water molecules [17]



A) Water molecules in a container outside the body move in constant random (Brownian) motion.
B) Highly cellular tissue impedes the movement of water molecules. These tissues may be intravascular, intracellular or extracellular.
C) Tissue with low cellularity or defective cells permits greater water molecule movement.

DWI has several benefits over other methods of investigation; it is a non-invasive technique, which does not require the use of contrast or exposure to radiation[18]. DWI scans are carried out rapidly, taking 5 minutes and can be added onto an existing MRI protocol without substantial increase in acquisition time[9].

DWI was initially used for the investigation of intracranial pathology including epilepsy and dementia[10]. Subsequent developments in technology facilitated its use for extracranial pathology and the potential for evaluation of tumours began to be investigated[9]. Applications of DWI in patients with cancer include tumour detection, tumour characterisation, distinguishing between tumours and benign lesions, monitoring treatment response and prediction of treatment response[10]. ADC has shown potential as a biomarker to predict and assess efficacy of chemotherapy and radiotherapy in a number of other cancers[11,19].

In recent years, the use of DWI in rectal cancer has been explored by a number of studies. A systematic review of the use of multiparametric MRI in rectal cancer found that, although there is some good evidence for the benefit of DWI in the

assessment of response to LCRT, the evidence for its usefulness, in terms of predicting response, remains unclear[15].

4.2 Aims

The aim of this study was to determine the potential ability of DWI volumetry and ADC measurement to predict and assess response to neoadjuvant LCRT for rectal cancer, in comparison with standard T2-weighted MRI volumetry and mrTRG, using histopathological response as the gold standard.

4.3 Methods

4.3.1 Patients

This was a single institution retrospective cohort study. Consecutive patients meeting the eligibility criteria between May 2008 and February 2015 were included. Inclusion criteria included 1) patients with biopsy-proven diagnosis of rectal cancer 2) discussed at colorectal multi-disciplinary team (MDT) meeting 3) undergoing neoadjuvant long-course chemoradiotherapy (LCRT) prior to restaging and then potential surgical resection 4) routine MRI (including DWI) staging pre- and post-LCRT. All patients were reviewed at the colorectal cancer MDT as per usual practice.

4.3.2 Treatment

All patients underwent neoadjuvant LCRT. External beam radiotherapy was given as 45 Gy delivered in 25 fractions (daily dose 1.8 Gy) over a five-week period and all patients completed the full course. Concomitant chemotherapy was given as the oral 5-FU derivative capecitabine at the dose of 825 mg/m^2 twice daily. One patient developed toxicity (chest pain) after one week and was changed to oxaliplatin, which was completed without difficulty. All patients underwent routine DWI as part of staging investigations prior to treatment and restaging scans were carried out 6-8 weeks after completion of neoadjuvant therapy. The surgical approach was decided following re-discussion of results at the colorectal MDT and resections were carried out according to the principles of total mesorectal excision.

4.3.3 Histology

Histology, specifically tumour regression grade (TRG), was used as the gold standard against which to compare the value of DWI/ADC as a diagnostic test. Tumour Regression Grades had been variably reported previously and therefore the TRG for all patients was reassessed. Histological reporting was carried out by an experienced Consultant gastrointestinal pathologist (Professor R Feakins) according to a standard protocol and TRG was assessed using the Royal College of Pathologists' favoured classification system (as advised by the Association of Coloproctologists Guidelines for the Management of Cancer of the Colon, Rectum and Anus) shown in Table 4.1[20]. For two patients histology slides were unavailable, one was staged post-operatively as T3N0(M1), stage 4 and the other as T3N1, stage 3, both were included within the TRG 4 group.

Patients were considered to be responders to treatment if their histology showed a TRG 1, indicating a pathological complete response (pCR) to LCRT. Non-responders were those classified as TRG 2, 3 or 4. Staging was reported according to the Union for International Cancer Control (UICC) staging system 7th edition[21].

Table 4.1 Classification of tumour regression grade (TRG) [20]

TRG	Finding on histology
TRG 1	No viable tumour cells (fibrosis or mucus lakes only)
TRG 2	Single cells or scattered small groups of cancer cells
TRG 3	Residual cancer outgrown by fibrosis
TRG 4	Minimal or no regression (extensive residual tumour)

4.3.4 MRI protocol

Scans were performed on a 1.5T Philips Achieva scanner (Philips Healthcare, Best, The Netherlands, Release 2.6) in conjunction with a sixteen-element body coil array. The study site protocol includes T2-weighted Turbo Spin Echo (TSE) axial and sagittal with a large field-of-view (FOV), T1-weighted TSE large FOV, DWI and oblique small FOV T2-weighted TSE or for larger tumours a 3D VISTA. DW images were obtained using a free-breathing multi-slice spin-echo (SE) echoplanar imaging (EPI) sequence with the following parameters: repetition time(TR) 5300 - 5800 ms, echo time (TE) 62ms, EPI factor 60, three averages, FOV 400 - 450mm, rectangular FOV 75%, acquisition matrix 112 ×256, 32 slices, slice thickness 6mm, slice gap 1mm.

Six motion probing gradients with b-values of 0, 100, 200, 500, 750 and 1000 s mm^{-2} were applied in three orthogonal directions and trace images were synthesised for each b-value using the mean of three orthogonal directions. *In-vitro* reproducibility was assessed on this scanner with a coefficient of variation of less than 2%[22].

The T2-weighted TSE axial images were obtained using the following acquisition parameters: FOV 375-450 mm, TE 100ms TR > 6000ms, TSE factor 15, acquired voxel size 1×1.3mm^2, acquisition matrix 368×195 reconstructed to 512×512, 6mm slice, 1mm gap, 32 slices. No intravenous or oral contrast mediums, rectal distension or bowel preparation were used. To limit artefact related to peristalsis, 20mg of hyoscine butylbromide (Buscopan, Boehringer Ingelheim, Germany) was routinely given intravenously at the start of the scan.

4.3.5 Image evaluation/analysis

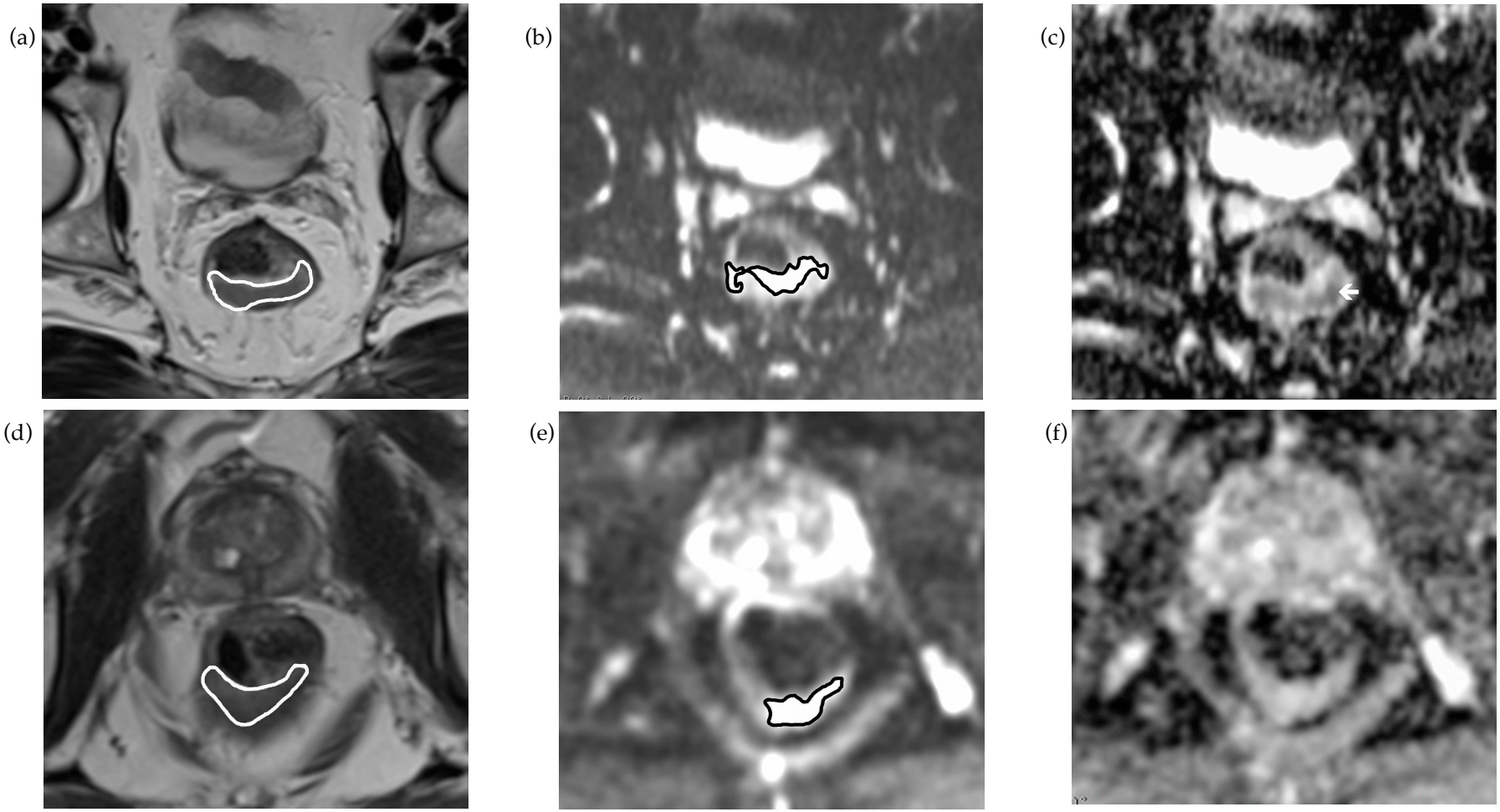
Images were analysed using commercially available software OsiriX (Pixmeo SARL, Bernex, Switzerland, version 8.5.2)[23]. T2-weighted and DW images were analysed by 2 readers (KL/Dr A Parsai) in consensus, using T2-weighted images as a reference when interpreting the DWI scans. Both readers were blinded to clinical history, histology results and MRI reports. Tumour contours were manually drawn on each axial slice and volumes were calculated automatically by the software. For the DWI scans, contours were drawn on the b=1000 images. ADC maps were automatically generated on the scanner using a mono-exponential fit on a pixel-by-pixel basis; b=0 was excluded in order to eliminate perfusion effects. ADC regions of interest (ROIs) were manually drawn in areas of tumour, corresponding to areas of high signal on the b=1000 images, see Figure 4.2. On the post-LCRT scans, where there was no residual tumour evident on DWI, ROIs were drawn at the former location of the primary tumour. T2-weighted images were used for assessment of mrTRG by a single Radiologist (Dr A Parsai).

4.3.6 Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS Inc. Chicago, USA, version 22.0) and a two-sided p value of <0.05 was considered statistically significant. Fisher's exact test was used to compare proportions for baseline categorical variables. As data is non-parametric, measures of location are expressed as median and non-parametric tests of significance were used.

The Mann-Whitney U test was used to compare responders and non-responders and the Wilcoxon signed-rank test was used to compare pre and post data for all patients. Percentage change between pre- and post-LCRT measurements (Δ) was calculated as 100×((post-pre)/pre). Spearman correlation analysis was used to assess the relationship between mrTRG and histological TRG.

Figure 4.2 MR images from a 63-year-old man classified as a non-responder (TRG 3) The white line on figures (a) and (d), and black line on figures (b) and (e), indicates the manual ROI drawn for calculation of tumour volume. (a) Pre-LCRT T2-weighted images (b) Pre-LCRT high b-value DW-MRI images and (c) Pre-LCRT ADC map – demonstrating restricted diffusion (darker areas) within the tumour (indicated by arrow). (d) Post-LCRT T2-weighted images (e) Post-LCRT high b-value DW-MRI images – with area of high signal intensity indicating residual tumour (f) Post-LCRT ADC map. ROI: region of interest; LCRT: long-course chemoradiotherapy; ADC: apparent diffusion coefficient.

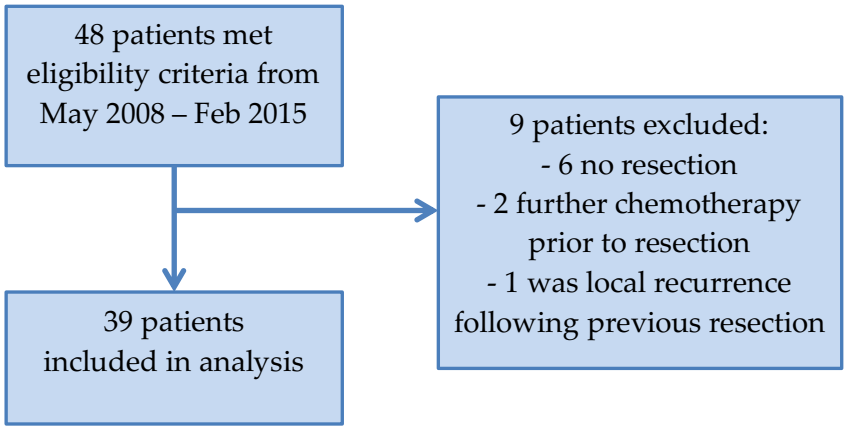


4.4 Results

4.4.1 Patients

48 patients met eligibility criteria between May 2008 and February 2015, see Figure 4.3. 9 patients were excluded: 6 of these had no operation carried out due to non-resectable disease, 2 were excluded as they underwent further chemotherapy following LCRT and 1 because the rectal cancer was a local recurrence following previous high anterior resection.

Figure 4.3 Flow chart showing patient inclusion.



4.4.2 Baseline data

39 patients were therefore included in the analysis, 30 men (76.9%) and 9 women (23.1%). The median age was 63 years (range 25-78). Staging according to the UICC classification, prior to LCRT, is shown in Table 4.2. Three patients were classified as stage 4 due to internal iliac nodal involvement in one, a superior rectal node in another and liver metastasis (resectable) in the third patient. 17 patients underwent an abdomino-perineal excision of rectum (APER), 20 underwent an anterior resection (3 with end colostomy) and 1 patient had a local excision.

Table 4.2 Patient characteristics. The other/none group under operation includes three patients with end colostomy (Hartmann's procedure) all in the non-pCR group. IQR: interquartile range; pCR: pathological complete response; LCRT: long-course chemoradiotherapy; APER: abdomino-perineal excision of rectum; UICC: Union for International Cancer Control staging system.

	pCR group (n = 5)	Non-pCR group (n = 34)	p-value
Female : Male	2 : 3	7 : 27	0.572
Median age (IQR) years	65 (62 – 75)	61 (53 – 72)	0.256
Pre-LCRT median T2 tumour volume cm^3 (IQR)	29.0 (20.1 – 38.6)	29.8 (20.5 – 58.3)	0.674
Pre-LCRT UICC stage 1 2 3 4	 1 0 3 1	 1 7 24 2	0.171
Operation APER Anterior resection Other / none	 2 2 1	 15 15 4	1.000
Mucinous	1 / 4 (25%) (1 not applicable)	10 / 33 (30.3%) (1 missing)	1.000

4.4.3 Results of staging and TRG

Comparing pre-LCRT staging with histological staging, 22/39 (56.4%) patients had disease downstaged with LCRT. 4 patients had a pathological complete response (pCR) following resection. One patient had a clinical and radiological complete response on re-staging following LCRT. This patient chose not to undergo surgery and has been intensively followed-up for over 5 years with no evidence of recurrence.

The patient with the clinical complete response was included with these to give an overall pCR rate of 5/39 (12.8%). The remaining 34 patients were classified as 'non-pCR'. Table 4.2 shows the characteristics of the two groups.

The results for pre- and post-LCRT (histological) staging, as well as histological TRG are shown in Table 4.3. The 4 patients with a pCR were classified as TRG 1, of the remaining patients 2 were TRG 2, 19 were TRG 3 and 13 were TRG 4. On histological staging, in addition to the 5 patients classified as pCR, 12 patients were stage 1, 6 were stage 2, 15 were stage 3 and 1 was stage 4 (liver metastasis, subsequently resected).

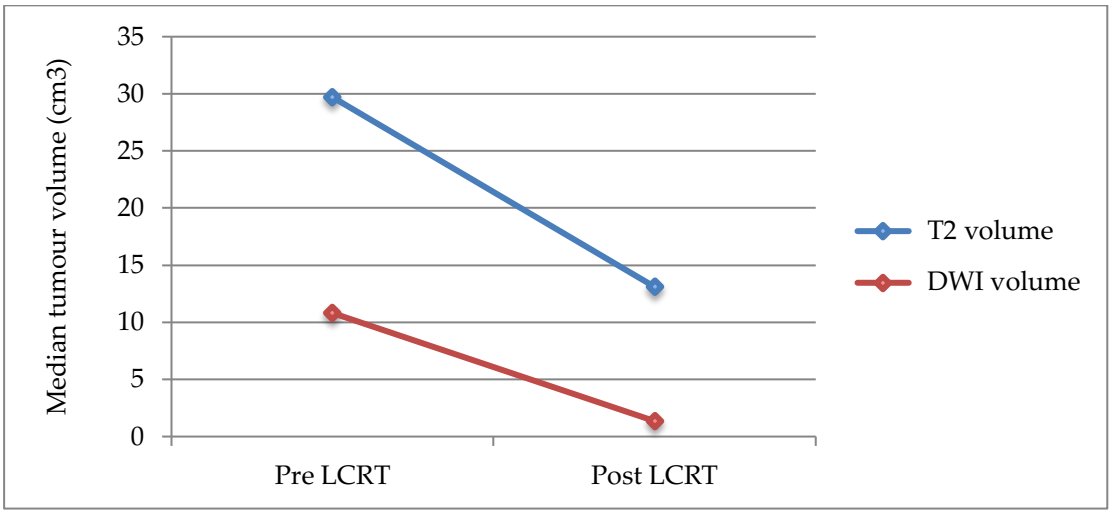
Table 4.3 Pre-/post-LCRT UICC staging and TRG. LCRT: long-course chemoradiotherapy; UICC: Union for International Cancer Control staging system; TRG: tumour regression grade; pCR: pathological complete response.

	pCR group (n = 5)	Non-pCR group (n=34)
Pre-LCRT stage		
1	1	1
2	0	7
3	3	24
4	1	2
Histological stage		
pCR	5	0
1	0	12
2	0	6
3	0	15
4	0	1
Histological TRG		
1	5	0
2	0	2
3	0	19
4	0	13

4.4.4 Wilcoxon signed-rank test: pre- and post-LCRT results for all patients

For all patients, there was a significant difference between the T2-weighted tumour volume, DWI tumour volume and ADC values on the pre-LCRT scan vs. post-LCRT scan ($p = <0.001$ for each). Volumes measured on DWI were significantly smaller than those measured on T2 imaging ($p = <0.001$). Figure 4.4 illustrates the difference between T2 and DWI volume measurements on pre- and post-LCRT imaging, indicating a reduction in tumour volume following LCRT.

Figure 4.4 Pre- and post-LCRT tumour volumes on T2 and DWI imaging for all patients. LCRT: long-course chemoradiotherapy.



4.4.5 Mann Whitney U test: pCR and non-pCR groups pre- and post-LCRT

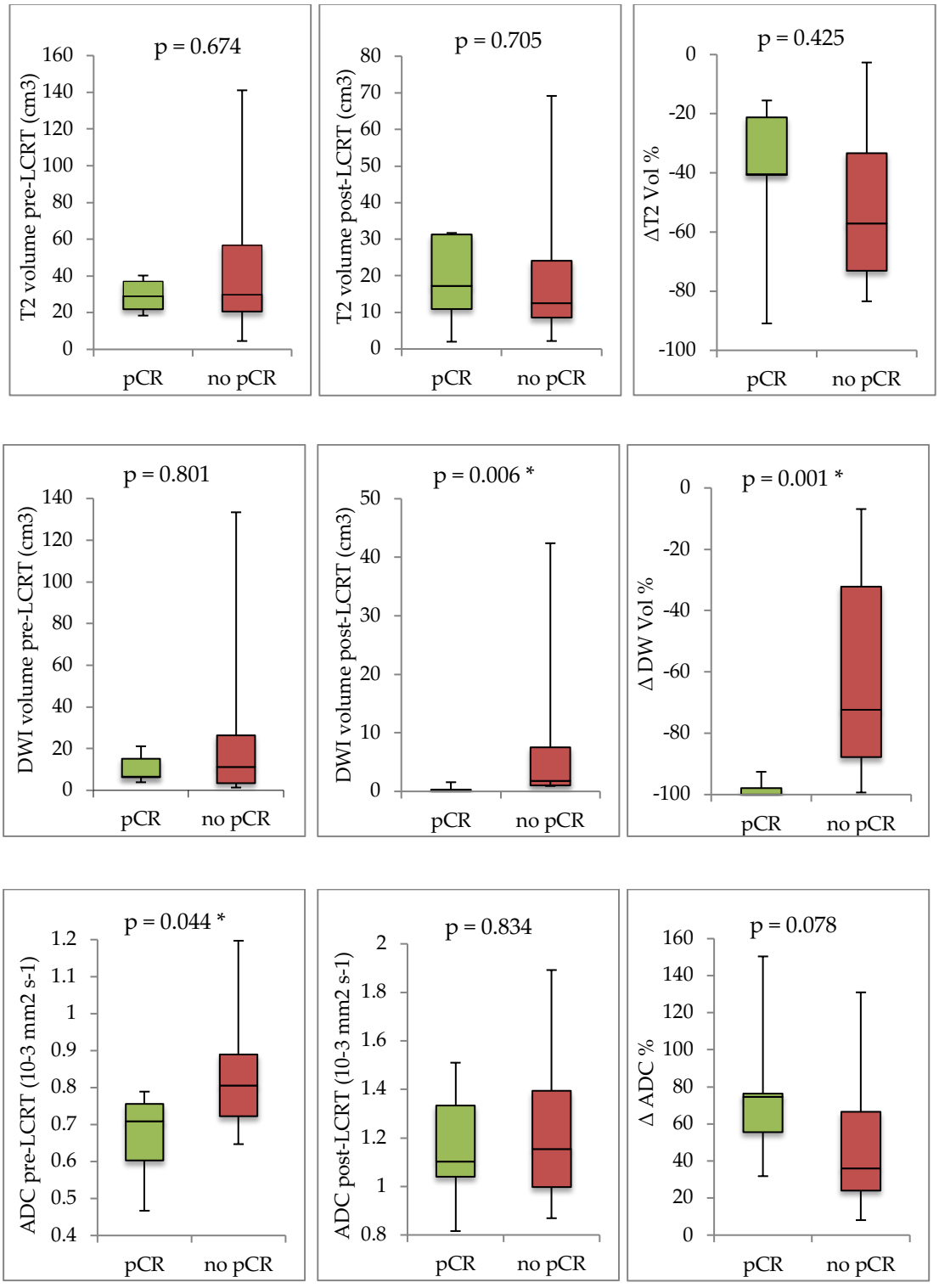
This analysis was done with the patients grouped into responders and non-responders according to pCR status. Table 4.4 shows the median values (and quartiles) for T2-weighted MRI volume, DWI volume, ADC values and mrTRG for three groups: all patients; pCR and non-pCR. Three sets of values are shown: pre-LCRT; post-LCRT and percentage change between these two measurements (except for mrTRG).

The results in Table 4.4 show that for T2-weighted MRI there was no significant difference between responders and non-responders on the pre, post or ΔT2 Volume %. On DWI there was no significant difference in the pre-LCRT images but significant difference between the pCR and non-pCR groups on the post-LCRT scan volume (0 vs. 1.8 cm^3, p = 0.006) and on ΔDW volume % (-100 vs. -72.3%, p = 0.001), see Figure 4.5. Figure 4.5 also illustrates the significant difference in pre-LCRT ADC values between responders and non-responders (0.7 vs. 0.8 × 10^{-3} $mm^2 s^{-1}$, p = 0.044). There was no significant difference between pCR and non-pCR on post-LCRT ADC or ΔADC values.

Table 4.4 Median volumes, ADC values and mrTRG for all patients, pCR and non-pCR groups. * indicates a significant p value. pCR: pathological complete response; ADC: apparent diffusion coefficient; mrTRG: MRI tumour regression grade; IQR: interquartile range.

	All patients n = 39 median (IQR)	pCR n = 5 median (IQR)	Non-pCR n = 34 median (IQR)	p-value
T2 volume				
Pre (cm^3)	29.7 (20.5 – 53.9)	29.0 (20.1 – 38.6)	29.8 (20.5 – 58.3)	0.674
Post (cm^3)	13.1 (8.3 – 24.5)	17.2 (6.5 – 31.5)	12.5 (8.3 – 24.4)	0.705
ΔT2 Volume (%)	-54.6 (-73.5 – -30.9)	-40.6 (-65.8 – -18.3)	-57.2 (-74.1 – -31.3)	0.425
DWI volume				
Pre (cm^3)	10.8 (3.9 – 25.4)	6.5 (5.1 – 18.1)	11.2 (3.0 – 27.7)	0.801
Post (cm^3)	1.4 (0.7 – 5.2)	0 (0 – 0.9)	1.8 (1.0 – 8.6)	0.006 *
ΔDW Volume (%)	-78.0 (-93.9 – -37.2)	-100 (-100 – -95.3)	-72.3 (-89.2 – -28.2)	0.001 *
ADC				
Pre (10^{-3} mm^2 s^{-1})	0.8 (0.7 – 0.9)	0.7 (0.5 – 0.8)	0.8 (0.7 – 0.9)	0.044 *
Post (10^{-3} mm^2 s^{-1})	1.2 (1.0 – 1.4)	1.1 (0.9 – 1.4)	1.2 (1.0 – 1.4)	0.834
ΔADC (%)	43.6 (24.2 – 74.2)	74.7 (43.6 – 113.4)	36.0 (23.8 – 68.6)	0.078
mrTRG				
Post	3 (2 – 4)	1 (1 – 2)	3 (3 – 4)	0.002 *

Figure 4.5 Difference in T2-weighted MRI volume, DWI volume and ADC values for pCR and non-pCR groups: pre-LCRT; post-LCRT and percentage change between these two measurements. The middle line in each box represents the median value, with the boundaries of the box representing the quartiles and whiskers showing minimum and maximum values. * indicates a significant p value. pCR: pathological complete response; LCRT: long-course chemoradiotherapy; ADC: apparent diffusion coefficient.



4.4.6 Mann Whitney U test: downstaged and non-downstaged groups pre- and post-LCRT

This analysis was done with the patients grouped into responders and non-responders according to downstaging following LCRT. Table 4.5 shows the median values (and range) for T2-weighted MRI volume, DWI volume and ADC values for three groups: all patients; downstaged patients and non-downstaged patients. As with the previous table, three sets of values are shown: pre-LCRT; post-LCRT and percentage change between these two measurements. There was no difference between patients whose tumours had been downstaged following LCRT and those whose tumours were not downstaged on any of the parameters measured.

Table 4.5 Median volumes, ADC values and mrTRG for all patients, downstaged and non-downstaged groups. IQR: interquartile range; ADC: apparent diffusion coefficient; mrTRG: MRI tumour regression grade.

	All patients n = 39 median (IQR)	Downstaged n = 22 median (IQR)	Non-downstaged n = 17 median (IQR)	p-value
T2 volume				
Pre (cm^3)	29.7 (20.5 – 53.9)	33.9 (24.4 – 47.1)	27.4 (11.1 – 59.1)	0.174
Post (cm^3)	13.1 (8.3 – 24.5)	13.9 (8.3 – 31.4)	11.9 (8.7 – 20.0)	0.497
ΔT2 Volume (%)	-54.6 (-73.5 – -30.9)	-55.6 (-78.1 – -35.7)	-52.1 (-70.2 – -19.9)	0.308
DWI volume				
Pre (cm^3)	10.8 (3.9 – 25.4)	15.7 (4.0 – 25.7)	9.0 (2.8 – 25.9)	0.444
Post (cm^3)	1.4 (0.7 – 5.2)	1.1 (0.3 – 6.8)	2.2 (1.1 – 6.5)	0.328
ΔDW Volume (%)	-78.0 (-93.9 – -37.2)	-79.5 (-97.3 – -39.7)	-72.0 (-89.4 – -19.3)	0.141
ADC				
Pre ($10^{-3}\,mm^2\,s^{-1}$)	0.8 (0.7 – 0.9)	0.8 (0.7 – 0.8)	0.9 (0.7 – 1.0)	0.051
Post ($10^{-3}\,mm^2\,s^{-1}$)	1.2 (1.0 – 1.4)	1.1 (1.0 – 1.3)	1.3 (1.0 – 1.5)	0.395
ΔADC (%)	43.6 (24.2 – 74.2)	44.4 (27.8 – 75.2)	33.9 (24.1 – 66.4)	0.497
mrTRG				
Post	3.0 (2.0 – 4.0)	3.0 (2.0 – 3.3)	3.0 (2.0 – 4.0)	0.386

4.4.7 Correlation analysis: mrTRG and histological TRG

A Spearman's rank order correlation run to determine the relationship between histological TRG and mrTRG showed a strong positive correlation, which was statistically significant, r_s = 0.787, p = <0.001. mrTRG showed sensitivity of 80% and specificity of 97% for assessment of TRG on post-LCRT images.

4.4.8 Mann Whitney U: Analysis of clinical and pathological variables

Pre-LCRT ADC values were significantly lower in those with negative nodes on histological analysis compared with those with positive nodes or systemic metastases, with either UICC stage 3 or 4 disease (0.750 vs. 0.861 10^{-3} mm^2 s^{-1}, p = 0.030).

Pre-LCRT ADC values were not significantly different in tumours with a mucinous subtype on histology vs. those without this subtype (p = 0.379), tumours with and without extra-mural venous invasion (p = 0.232) and in patients who had a subsequent recurrence of their cancer (p = 0.157). Pre-LCRT ADC values also did not depend on gender (p = 0.548) or age (below or above median) (p = 0.077). These findings are summarised in Table 4.6.

Table 4.6 Pre-LCRT ADC values and clinicopathological variables. *indicates a significant p-value. The patient with a complete clinical response was counted within N0 but was not applicable for other pathological variables. ADC: apparent diffusion coefficient; T: tumour; N: nodes; M: metastases; EMVI: extra-mural venous invasion.

Clinico-pathological variable	Groups	Number of patients	Median ADC (IQR) (10^{-3} mm^2 s^{-1})	p-value
Gender	Male	30	0.788 (0.697 – 0.870)	0.548
	Female	9	0.774 (0.727 – 0.945)	
Age	<63 (median)	19	0.816 (0.736 – 0.920)	0.077
	>/= 63	20	0.750 (0.686 – 0.840)	
Histological T stage	T0-2	20	0.755 (0.703 – 0.829)	0.339
	T3-4	19	0.820 (0.711 – 0.900)	
N/M stage	N/M 0	23	0.750 (0.687 – 0.825)	0.030 *
	N/M 1	16	0.865 (0.740 – 0.958)	
Mucinous tumour	Non-mucinous	26	0.770 (0.709 – 0.870)	0.379
	Mucinous	11	0.787 (0.753 – 0.975)	
	Unknown or n/a	2	-	
EMVI	No EMVI	31	0.774 (0.710 – 0.840)	0.232
	EMVI present	6	0.917 (0.721 – 1.097)	
	Unknown or n/a	2	-	
Recurrence	No recurrence	26	0.753 (0.697 – 0.825)	0.157
	Recurrence	13	0.846 (0.750 – 0.895)	

4.5 Discussion

DWI volumetry and ADC measurements were evaluated in the prediction and assessment of response to neoadjuvant LCRT. The DWI protocol was introduced into the routine staging investigations of patients with rectal cancer at this institution. Analysis of DWI parameters was carried out without difficulty, although this would require some additional time in comparison with a standard MRI report. This demonstrates that the use of DWI measures would be feasible in routine clinical practice.

Measures derived from DWI show the potential to be useful for the prediction of response to LCRT. Pre-LCRT ADC values were lower in the pCR group compared with the non-pCR group. This finding is in keeping with several previous studies[16,18,24,25] and provides further evidence of the potential use of DWI as a tool for prediction of response to LCRT. These findings have been replicated in other tumour types[26-28]. Biologically, the explanation for why ADC values should be lower in subsequent responders is that the higher ADC in non-responding tumours reflects areas of tumour necrosis, which leads to hypoxia-mediated resistance to therapy[16,19]. There remains conflicting evidence in this area, as other studies have shown no difference in pre-LCRT ADC values[29-31], or even contradictory findings of higher pre-LCRT ADC values in responders[32,33].

This study showed a difference on DWI volumetry between the pCR and non-pCR groups on the post-LCRT scan and on ΔDW volume %. Results for T2-weighted volumetry were not significant and this is not surprising, as standard T2-weighted MRI has previously been found to overestimate tumour volume[34] and overstage rectal cancers [7]. Other studies have found similar results for the improved assessment of tumours post-LCRT using DWI volumetry[35-37]. It has been suggested, however, that with increasing experience of the interpreting radiologist, the benefit of DWI over T2-weighted MRI is reduced[38].

mrTRG showed a high level of sensitivity and specificity in terms of assessing TRG on the post-LCRT scans. Unlike DWI, use of mrTRG is inherently limited to the assessment of response. It has been shown to be the most reliable current method to assess response prior to surgery with potential as a biomarker to stratify treatment[38].

A systematic review of the role of DWI in rectal cancer, published in 2014, concluded that DWI is not yet accurate enough to safely predict complete response with a low overall PPV of 54% and accuracy of 68-72% on pre-LCRT imaging[14]. The authors concluded that the major strength of DWI lies in identification of non-responders. One of the reasons for this finding was heterogeneity between studies[14]. Several authors have proposed reasons for the discrepancies between the results of individual studies. These include variations in the definition of response to treatment; technical parameters including MRI protocol and b-values used; study populations and ADC measurement techniques including ROI size and positioning[15,25,40].

A number of studies have compared qualitative assessment of DWI images with quantitative measurements and found good results for qualitative interpretation[38,41-43]. A pooled analysis of these data showed a specificity of 94% and accuracy of 87%[14]. This could perhaps offer a less time-consuming option for routine practice[44].

The use of DWI in rectal cancer imaging is becoming widespread and its routine use in assessment following LCRT is now recommended by international guidelines[45]. Other novel imaging techniques have been explored for use in assessing rectal cancer, including perfusion or dynamic contrast-enhanced MRI and positron emission tomography-computed tomography (PET-CT). These have limitations; it is difficult to differentiate between residual tumour and inflammation on PET-CT, it is not possible to determine nodal status and extra scans are required with exposure to radiation[11,46]. The applicability of perfusion MRI is limited by the lack of evidence and variations in techniques limiting reproducibility[15].

Although in this study, as in many others, response was classified into pCR and non-pCR according to TRG, in reality there is a spectrum of response, with some partial responders. Studies assessing DWI have defined response using a number of different methods including tumour shrinkage, downstaging, and variations in the systems used to assess histological TRG. In this study pCR was used to define response because this is the most clinically relevant definition. Patients with pCR are those who have gained the greatest benefit from LCRT and could potentially avoid surgery altogether if a 'watch and wait' policy were followed. The analysis was also carried out grouping patients into those with downstaging of their tumour following LCRT, and those without downstaging but there was no significant difference found between these groups on any of the measures. Downstaging is inherently reliant on

the accuracy of pre-LCRT staging, including assessment of nodes, it has less clinical relevance than complete response and is a less objective endpoint.

An interesting finding of this study was that ADC values pre LCRT were lower in those with negative nodes. This is despite the fact that the pCR and non-pCR groups had similar staging pre-LCRT. Two previous studies, which assessed the ADC values in responders and non-responders found a conflicting result of lower ADC values pre-LCRT in node positive patients[33,47]. Determination of whether DW-MRI measurements can differentiate between favourable and non-favourable histological characteristics, beyond just TRG, needs further studies.

Previous studies have investigated the use of interim imaging at various time periods during LCRT. Early assessment with a DWI scan a few weeks into treatment has shown promising results for stratifying patients into responders and non-responders[24]. This remains an area of ongoing study but interim scanning would have significant logistical and financial implications for routine practice and might add further complexity into the decision-making process. The timing of post-LCRT assessment is also important, with recent evidence showing that an increased time period of 10-11 weeks can improve response rates compared with the standard 6-8 weeks[48].

Limitations of this study include its single-centre, retrospective nature with fairly small numbers although selection bias was avoided by including consecutive eligible patients. All patients with rectal cancer who had MRI during this time period also had DWI scanning, so this was not a reason for reduced numbers. There were multiple other reasons for the low patient numbers achieved in the study. Although this is a busy colorectal unit, there were proportionally fewer rectal cancers (vs. colorectal cancers) during this time period than expected. Fewer patients underwent resection than anticipated, for several reasons, including that patients either had inoperable disease or metastatic disease. The unit is a tertiary referral centre, and because of this, some patients undergoing resection had their staging investigations in a separate NHS Trust. The pCR rate in this patient sample was also slightly lower than might be expected (12.8% in this sample vs. 16% in a large published pooled analysis[2]). There were some patients excluded for other reasons, as documented in Figure 4.3. In combination, these factors led to smaller sample size and particularly a smaller pCR group than would have been desired or ideal. This is likely to lead to type II errors and limited subgroup analysis.

Other limitations of the study include that scans were carried out pre- and post-LCRT without an interim scan, this is in keeping with routine clinical practice in the UK. This study used two readers in consensus rather than independently, however previous studies have shown no significant inter-observer variation with strong agreement in measuring tumour volume and ADC values[29,36]. ADC measurements were derived from a sample ROI, which may not be representative of the whole tumour sample, especially considering tumour heterogeneity. Sample ROI was used to reflect normal practice as outlining of the whole tumour to calculate ADC would be inefficient for everyday use[35]. ADC measurements were not used to assess nodal staging as this was beyond the scope of the study; qualitative analysis was also not used.

Incorporating DWI measurements into routine clinical decision-making will require consensus of MRI protocols[24] and standardisation of techniques including hardware, software and analysis methods[15]. These are all factors that affect image quality and interpretations. Recent consensus guidelines on the use of DWI have been published for the first time[19]; large multi-centre prospective studies using this guidance will be needed to determine consistent threshold values. Use in clinical practice is complicated; radiologists need experience interpreting images in order to make an efficient assessment. Methods to improve efficiency are being developed, including semi-automated techniques for volume calculation[49]. At present, DWI remains one tool amongst many in the assessment of rectal tumours. The ultimate aim, of personalised therapy, may well require integration with other tools such as molecular biomarkers[14].

4.6 Conclusion

Incorporating DWI into routine clinical practice is feasible and measures derived from DWI show potential as non-invasive biomarkers for predicting and assessing response to LCRT in rectal cancer. Specifically, pre-LCRT ADC values calculated from DWI scans may be useful for the prediction of response to neoadjuvant LCRT. DWI, in terms of post-LCRT volume and ΔDW volume appears superior to T2-weighted MRI volumetry in assessing whether patients have had a complete response to LCRT. Following further research, biomarkers derived from DWI could possibly be used to rationalise the use of LCRT, a major risk factor for poor functional outcome.

4.7 References

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Chapter 5. Identifying molecular targets of response to neo-adjuvant chemoradiotherapy

5.1 Introduction

Traditionally, the complex treatment decision about which patients should undergo chemoradiotherapy (CRT) is determined by using a combination of anatomical, radiological, functional and patient-specific factors. In the UK, this happens in the setting of a colorectal multidisciplinary team (MDT) meeting. Molecular biomarkers, which could help to predict and stratify tumour response in the algorithm would improve patient selection and help to inform management decisions. These would help the move away from protocol-driven care towards individualised cancer therapy.

A biomarker for the prediction of response to neoadjuvant CRT would potentially allow patients with operable tumours, who were predicted to have a poor response to CRT to proceed straight to surgery. Neoadjuvant CRT is a major risk factor for poor functional outcome[1] and its use would ideally be limited to those patients who would benefit from it on an individual level. In a locally advanced tumour where the circumferential margin is involved, there is a risk of incomplete resection with primary surgery. If it was possible to predict poor response to neoadjuvant therapy then potentially early systemic therapy would be of greater benefit to reduce the risk of metastatic disease.

In tumours that are sensitive to neoadjuvant therapy, a biomarker predicting this response, would introduce the option to increase the dose, aiming for complete response and organ preservation. For patients with highly radiosensitive and chemosensitive tumours, especially the cohort who particularly wish to avoid surgery, there would be an option of intensifying the neoadjuvant regime with the aim of avoiding surgical intervention and following a treatment protocol more akin to that for anal carcinoma. The effect on sphincter function of an intensified radiotherapy dose without subsequent surgery is not fully known but it is likely to be superior to that of the combined effect of an anterior resection and CRT.

The introductory chapter discusses a number of molecular biomarkers, which have been investigated as markers of response to neoadjuvant therapy in rectal cancer.

Those discussed make up a small proportion of the potential markers that have been studied. Response to therapy is complex, involving multiple pathways and, as such, it is unlikely that any one marker can entirely predict response in all cases[2]. The first step in developing molecular biomarkers is an improved understanding of the mechanisms behind response and especially non-response to therapy. This involves an improved understanding of which elements of a tumour's microenvironment and metabolism contribute to the resistance to CRT.

Molecular biomarkers that have been investigated have either been present in tumour tissue or blood[3]. Potential biomarkers from within tumour tissues include specific gene mutations[4]; methylation profiles[5]; combined gene expression profiles[6]; expression of specific proteins or metabolites[7]; and elements of the tumour immune microenvironment[2]. Biomarkers taken from blood again include expression of proteins and metabolites, for example carcinoembryonic antigen (CEA)[8]; markers of the host immune response[9], circulating tumour cells[10] and nucleic acids[11]. DNA alterations, including chromosomal alterations and instability[12], and also single nucleotide polymorphisms have also been investigated[13].

Hypoxia has been identified as a key factor accounting for the differing sensitivity of individual tumours to radiotherapy and chemotherapy[14] and is a common cellular process linked to many of the biomarkers identified above[15]. It is present in over 50% of rectal cancers and affects the characteristics of the tumour as well as the response to therapy[16]. Hypoxia plays a key role in tumour growth and progression; hypoxic tumours are more resistant to radiotherapy since oxygen free radicals are required for the generation of DNA damage, such as DNA double strand breaks, caused by ionizing radiation[17]. Hypoxic tumours are also resistant to chemotherapy via a number of mechanisms. Some chemotherapeutic agents depend on cellular oxygenation for their mechanism of action; others act on tumour cells during DNA synthesis and are made less effective by the slow cell cycling occurring as a result of hypoxia[18]. Because of these links between hypoxia and response to CRT, markers of hypoxia may be useful as biomarkers of response to CRT.

MicroRNAs are small non-coding RNAs, 18-25 nucleotides in length that act as post-transcriptional regulators of gene expression[19]. They have an important functional role in cancer as they regulate oncogenes, tumour suppressor genes and genes responsible for processes including differentiation, invasion and dissemination[20]. Aberrant expression of microRNAs has been shown in many different types of

cancer, including colorectal cancer[21]. Distinct expression profiles have been linked to cancer prognosis or disease progression and can be used to classify cancers[22]. MicroRNAs have shown potential to predict sensitivity to anticancer treatment and influence sensitivity to chemotherapy and radiotherapy; this explains their potential as both biomarkers and targets for therapy[23].

MicroRNAs have been studied in several different diagnostic and prognostic aspects of rectal cancer. Their use in diagnosing rectal cancer has been studied in both tissue[24] and blood samples[25]. The possibility of using microRNAs to identify specific tumour subtypes, for example those that have CpG Island Methylator Phenotype positivity or TP53 mutations[26] has also been investigated. Gaedcke *et al.* have shown that expression of miR-135b correlates significantly with disease-free and cancer-specific survival[24] and other studies have considered the possibility of diagnosis of nodal positivity[27], liver metastates[28] or the likelihood of recurrent disease[29]. With regard to neoadjuvant therapy, microRNAs have been assessed in monitoring of response during CRT[30], assessing response following CRT[31] and even prediction of side-effects to radiotherapy[32], including fibrosis of the anal sphincters[33]. Despite the wide range of aspects investigated, compared with similar studies looking at colon cancer, there are relatively smaller numbers investigating rectal cancer, with fewer patients. Little overlap between identified microRNAs has been found, even in studies with similar methodology[34].

In rectal cancer, microRNAs have been shown to have potential as predictive biomarkers of response to neoadjuvant CRT. A number of studies have conducted arrays using pre-treatment tissue from biopsies to identify microRNAs with predictive potential[35-42]. These have shown differing results with little overlap in the microRNAs identified. Only four of these studies carried out validation of their results on a second group of patients[38,39,41,42] and none have used external validation. A further group of researchers quantified two specific microRNAs, and found that miR-21 predicted good response to therapy and miR-31 predicted poor response[43,44]. The largest study carried out so far identified five microRNAs from the literature which they quantified in pre-treatment biopsies from an initial group of 55 patients, followed by validation in a further group of 130 patients[45].

Three of these studies identified miR-21 as being differentially expressed in responders compared with non-responders to neoadjuvant therapy in rectal cancer[39,43,45]. Of these three studies, Lopes-Ramos *et al*. found it to be overexpressed in

responders[39], Carames *et al*. found it to overexpressed in non-responders[43] and Eriksen *et al*. found it to be overexpressed in responders in their test cohort and non-responders in their validation cohort[45], so the results are still somewhat conflicting. miR-21 has already been identified as a potential diagnostic and therapeutic target in colorectal cancer[46]. It is overexpressed in precancerous adenomas indicating that it may be involved in the progression to cancer at an early stage[47]. miR-21 has been previously shown to be involved in adaptations related to hypoxia in tumours[48], with overexpression allowing cells to avoid apoptosis in hypoxia and inhibition of miR-21 increasing cellular susceptibility to hypoxia[49]. Other miRs identified as overexpressed in responders in more than one study include miR-630[35,38], miR-223[38,40] and miR-1246[39,40]. One study identified eleven microRNAs as upregulated in complete responders and two that were down-regulated; the authors found that two microRNAs, miR-622 and miR-630 had 100% sensitivity and specificity in identifying tumour regression grade (TRG) 1 cases[35].

To date, no individual microRNAs or microRNA profiles have been validated for use as biomarkers in clinical practice, for the prediction of response to neoadjuvant CRT in rectal cancer.

5.2 Hypothesis and aims

The hypothesis of this study is that tumours in patients who respond to neoadjuvant therapy in rectal cancer express a different microRNA profile to those of non-responders.

The aim of the study is to identify microRNA targets with potential use as predictive biomarkers of response or non-response to neoadjuvant CRT in rectal cancer.

5.3 Methods:

5.3.1 Rectal cancer database

This study used data from an established Colorectal Cancer Tissue Bank database, which had been collected from 1998 to 2015 (ongoing). This computerised database contained prospectively recorded data from over 500 patient-anonymised samples.

Samples in the database had initially been collected at the time of operation and were paired normal and tumour tissues samples. From 2009 onwards, diagnostic samples were also taken at EUA and colonoscopy. All tissue was frozen immediately in liquid nitrogen upon resection from the specimen and stored at -80 °C. It was stored in compliance with the Human Tissue Act. Ethical approval was granted by the East London Research Ethics Committee, reference number: 09/H0703/106. This approval included access to the pathology archives at The Royal London Hospital to retrieve pathology blocks for these patients.

Using the Colorectal Cancer Tissue Bank database, a new database including all rectal cancers was created for this study. This included the data shown in Box 5.1. Details of neoadjuvant chemotherapy and radiotherapy were included.

5.3.2 Treatment

Box 5.1. Variables collected for database of rectal cancer patients

Demographics: age, gender
Date of surgery and type of procedure
Details of neoadjuvant and adjuvant therapy
Details of imaging carried out
Pre-operative radiological staging
Pathological report details:
- TNM stage
- Resection margin status
- Nodal status
- Extra-mural venous invasion status
- Histological tumour regression grade

Local and systemic recurrence
Disease free and overall survival

Some patients underwent short course radiotherapy (SCRT) with external beam radiotherapy given as 25Gy delivered in 5 fractions (daily dose 5Gy) over a five-day period. Other patients had long-course chemo-radiotherapy (LCRT) with external beam radiotherapy given as 45 Gy delivered in 25 fractions (daily dose 1.8 Gy) over a five-week period and concomitant chemotherapy given as the oral 5-FU derivative capecitabine at the dose of 825 mg/m^2 twice daily. All patients subsequently underwent surgical resection of the rectal tumour with histological analysis of the specimen. Staging was reported according to the Union for International Cancer Control (UICC) staging system 7th edition[50].

5.3.3 Classification of response

Data for tumour regression grade (TRG) had been variably and inconsistently reported previously and therefore the TRG for all patients who had undergone neoadjuvant therapy was reassessed. Histological reporting was carried out by an

experienced Consultant gastrointestinal pathologist (Professor R Feakins) according to a standard protocol and TRG was assessed using the Royal College of Pathologists' favoured classification system (as advised by the Association of Coloproctologists Guidelines for the Management of Cancer of the Colon, Rectum and Anus) shown in Table 5.1[51].

Table 5.1 Classification of tumour regression grade (TRG) [51]

TRG	Finding on histology
TRG 1	No viable tumour cells (fibrosis or mucus lakes only)
TRG 2	Single cells or scattered small groups of cancer cells
TRG 3	Residual cancer outgrown by fibrosis
TRG 4	Minimal or no regression (extensive residual tumour)

A cohort of responders and non-responders was required for the study. All patients in the database who had undergone CRT were therefore classified into one of three categories:

- Responders either had a TRG of 1-3 or were downstaged from pre-treatment staging to post-operative histological staging
- Non-responders were TRG 4 and had worsening or no change in their staging
- Intermediate: Insufficient data to determine response or conflict between TRG and change in staging

5.3.4 MiRNA extraction

Pre-treatment biopsy tissue blocks were identified for responders and non-responders. Tissue from prior to treatment was required as it is likely that neoadjuvant chemotherapy and radiotherapy would alter the expression of microRNAs in the resection specimen. Formalin fixed paraffin embedded (FFPE) tumour tissue sections on haematoxylin and eosin (H&E) stained slides were reviewed by a Consultant gastrointestinal pathologist (Professor R Feakins) who marked with a thin pen on each slide to show which biopsy contained cancer, an example of two slides is shown in Figure 5.1. These images were then used as a guide to which biopsy to use when extracting RNA.

Slides were cut and H&E stained by the Pathology Department (Royal London Hospital). Ten sections, each of 5 μm were used, giving a final depth of 50 μm

(0.05mm), to maximise yield of RNA as recommended by the protocol. The miRNeasy FFPE kit (Qiagen, UK) was used to purify total RNA from FFPE sections. Xylene was used for deparaffinisation, followed by 100% ethanol to extract residual xylene. Samples were then incubated in a lysis buffer containing proteinase K to release RNA from the sections. DNase treatment was used to eliminate genomic DNA. Ethanol was used to allow total RNA including microRNA to bind to the membrane of an RNeasy MinElute spin column and contaminants were washed away. RNA was then eluted in 20 µl RNase-free water. A NanoDrop™ Spectrophotometer (Nano-Drop Technologies, USA) was used to determine the concentration of RNA and the ratio of absorbance at 260 nm and 280 nm. Samples were stored at -80 °C.

Figure 5.1 Biopsy slides with marked areas of cancer indicated by orange circles

5.3.5 MicroRNA array

MicroRNA array profiling was carried out by Exiqon at Exiqon Services in Denmark. 28 samples were used, 11 from responders and 17 from non-responders. 325 ng total RNA from each sample was labelled with a fluorescent label. These, along with a fluorescent labelled reference RNA sample were mixed pair-wise and hybridized to the miRCURY LNA™ microRNA Array 7th Gen (Exiqon, Denmark). This array contains capture probes targeting all microRNAs in the miRBASE 19.0.

Hybridization was performed according to the miRCURY LNA™ microRNA Array Instruction manual. The miRCURY LNA™ microRNA array slides were scanned using the Agilent G2565BA Microarray Scanner System (Agilent Technologies, Inc., USA) and image analysis was carried out using the ImaGene 9.0 software (BioDiscovery, Inc., USA). For microRNA array data, normalisation was performed based on the average of the assays detected in all samples[52].

5.3.6 Technical validation

For technical validation of the results, the same 28 samples were used. In addition, samples were sent from 3 rectal cancer cell lines (HT55, SW837 and VACO4s) grown under three different oxygen conditions (0.2% oxygen, 1% oxygen and 20.9% oxygen) for 48 hours. These samples were provided by Anke Nijhuis, a member of our lab group. The rectal cancer cell lines were originally a gift from Professor Ian Tomlinson (Wellcome Institute for human genetics, University of Oxford).

Box 5.2 MicroRNAs selected for validation

* indicates those chosen based on p-values from array results

miR-574-3p *	miR-92b-3p *	miR-495-5p
miR-4539 *	miR-330-3p *	miR-221-5p
miR-4303 *	miR-23c *	miR-192-5p
miR-212-3p *	miR-215-5p *	miR-146b-5p
miR-1914-5p *	miR-483-5p *	miR-29c-3p
miR-4686 *	miR-210-3p	miR-342-5p
miR-505-5p *	miR-422a	miR-451a
let-7b-3p *	miR-767-5p	miR-29b-3p
miR-197-3p *	miR-206	

Technical validation of microRNA expression in pre-treatment tumour biopsies was carried out using real-time quantitative polymerase chain reaction (qPCR). A panel of 26 functionally relevant microRNAs was selected for validation; see Box 5.2 for the list of microRNAs selected. The majority of these (indicated by *) were selected based on p-values from the microRNA array results (see Table 5.5 for p-values) and the remainder were selected based on their link to colorectal cancer or response to chemo/radiotherapy from the literature, as follows:

- miR-422a expression levels are reduced in colorectal cancer and have been shown to correlate with stage of colorectal cancer[53]. miR-422a is also associated with relapse in gastric[54] and hepatocellular cancers[55] and linked to response to chemotherapy in osteosarcoma[56].
- miR-767-5p is involved in oncogenesis, is expressed in several cancers and has functional links to the miR-29 family[57] (miR-29b and miR-29c were also included in the technical validation).

- miR-206 is a tumour suppressor, attenuates tumour invasion, proliferation and migration in colorectal cancer and has been suggested as a potential therapeutic target[58,59].
- miR-495-5p is upregulated in radiotherapy sensitive lung cancer[60], and altered expression is also associated with multidrug resistance to chemotherapy[61].
- miR-210-3p is upregulated in hypoxia in a number of cancers[62]. It is also increased in hypoxic areas of colorectal cancer tissues and consistently upregulated in colorectal cancer cell lines grown in hypoxic conditions[63].
- miR-192-5p influences 5-fluorouracil resistance through cell cycle-mediated mechanisms[64] and was also shown to be significantly dysregulated in chemotherapy resistant oesophageal cancer cell lines[65].
- miR-29b-3p is known to critically affect cancer progression by functioning as a tumor suppressor[66]. Significantly altered expression was identified in non-responders to neoadjuvant chemoradiotherapy in rectal cancer[37]. Expression was an independent prognostic factor for disease-free survival, lymph node metastasis and pathological T stage classification in colorectal cancer[67].
- miR-221-5p is an oncogenic microRNA that is upregulated in several cancers, including colorectal[68]. Anti-miR-221 has been shown to sensitise human colorectal carcinoma cells to radiation[69] and miR-221 is also involved in radiotherapy resistance in glioblastomas[70] and chemoresistance in breast cancer[71].
- miR-146b-5p expression shows significant correlation with nodal stage in rectal cancer[72] and also regulates cell growth, invasion, and metabolism in colorectal cancer[73].
- miR-29c-3p expression is significantly decreased during early relapse in stage II and III colorectal cancer, and overexpression inhibited cell proliferation and migration[74]. miR-29c also enhances sensitivity to chemotherapy and radiotherapy in nasopharyngeal carcinoma[75].
- miR-342-5p has been shown to inhibit colorectal cancer cell proliferation and invasion[76]. Expression in plasma was found to be significantly different in responders and non-responders to radiotherapy for rectal cancer[77].
- miR-451a shows decreased expression in non-responders to neoadjuvant CRT in rectal cancer[40]. Expression is also decreased in gastric and colorectal cancer versus non-cancerous tissues and overexpression reduces cell proliferation and increases sensitivity to radiotherapy[78].

microRNA real-time qPCR was carried out by Exiqon at Exiqon Services in Denmark. Each RNA sample was reverse transcribed into complementary DNA

(cDNA) and run on the miRCURY LNA™ Universal RT microRNA PCR Custom Panel for the pre-determined microRNAs. Each microRNA was assayed once using ExiLENT SYBR® Green master mix. The results were background corrected[79] and normalised using the quantile normalisation method, which is recommended for this type of analysis[80].

5.3.7 Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS Inc. Chicago, USA, version 22.0). R (version 3.4.4) was used to create microRNA heatmaps and XLSTAT (version 14.7) was used to create volcano plots. A two-sided p-value of <0.05 was considered statistically significant. Fisher's exact test was used to compare proportions for clinical and pathological categorical variables. A moderated independent t-test was used to compare expression values in responders and non-responders; the moderation takes into account the variance of the whole data set. For microRNA array results p-values were adjusted for multiple testing by the Benjamini-Hochberg correction. Fold changes were calculated using the $2^{-\Delta\Delta CT}$ method normalised to endogenous control microRNAs[81].

Receiver-operating characteristic (ROC) curves were constructed, with calculation of the area under the curve (AUC) and determination of optimal cut-offs (with equal weighting to sensitivity and specificity) using the Youden index. These were used to determine sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and accuracy with Clopper-Pearson exact 95% confidence intervals.

Binary logistic regression was used to determine the proportion of responders/non-responders correctly predicted using selected microRNAs. miRWalk database version 3.0[82] and miRDB[83] were used to identify possible target genes. One-way ANOVA was used to compare expression levels between the three different oxygen tension groups, 0.2% oxygen, 1% oxygen and 20.9% oxygen used for colorectal cancer cell line treatments.

5.4 Results

5.4.1 Patients

The Colorectal Cancer Tissue Bank database was used to create a new database that comprised of all patients with rectal cancer; this began in May 1998 and up to the point of data collection in May 2015, 162 patients were included. The rectal cancer database included 100 male (61.7%) and 62 female (38.3%) patients; the age range was from 19 to 91 years with a median age of 67 years (interquartile range 61-73). The median age for men and women was similar at 67 and 68 years respectively. 55 patients underwent neoadjuvant therapy prior to surgical resection of their tumour, 27 had SCRT, 27 LCRT and 1 had chemotherapy only. The median age of patients having neoadjuvant therapy was also 67 years, and 74.5% of patients were male. The majority of patients (63.0%) were of White British ethnicity; the second most frequent ethnicity was Bengali (9.9%). Anterior resection was the most frequently carried out surgical resection (116 patients, 71.6%), 23 of these patients had an end colostomy created without anastomosis, APER was the next most frequent operation (21.6%) and 4 patients in the database underwent synchronous liver and colonic resection.

There were some difficulties encountered collecting biopsy specimens, as slides from the pathology archive at Barts Health NHS Trust are held off-site for all slides collected more than five years previously. Figure 5.2 shows patient inclusion and reasons for patients being excluded. Table 5.2 shows the details of the cohort of responders and non-responders identified.

Figure 5.2 Flow chart of patient inclusion

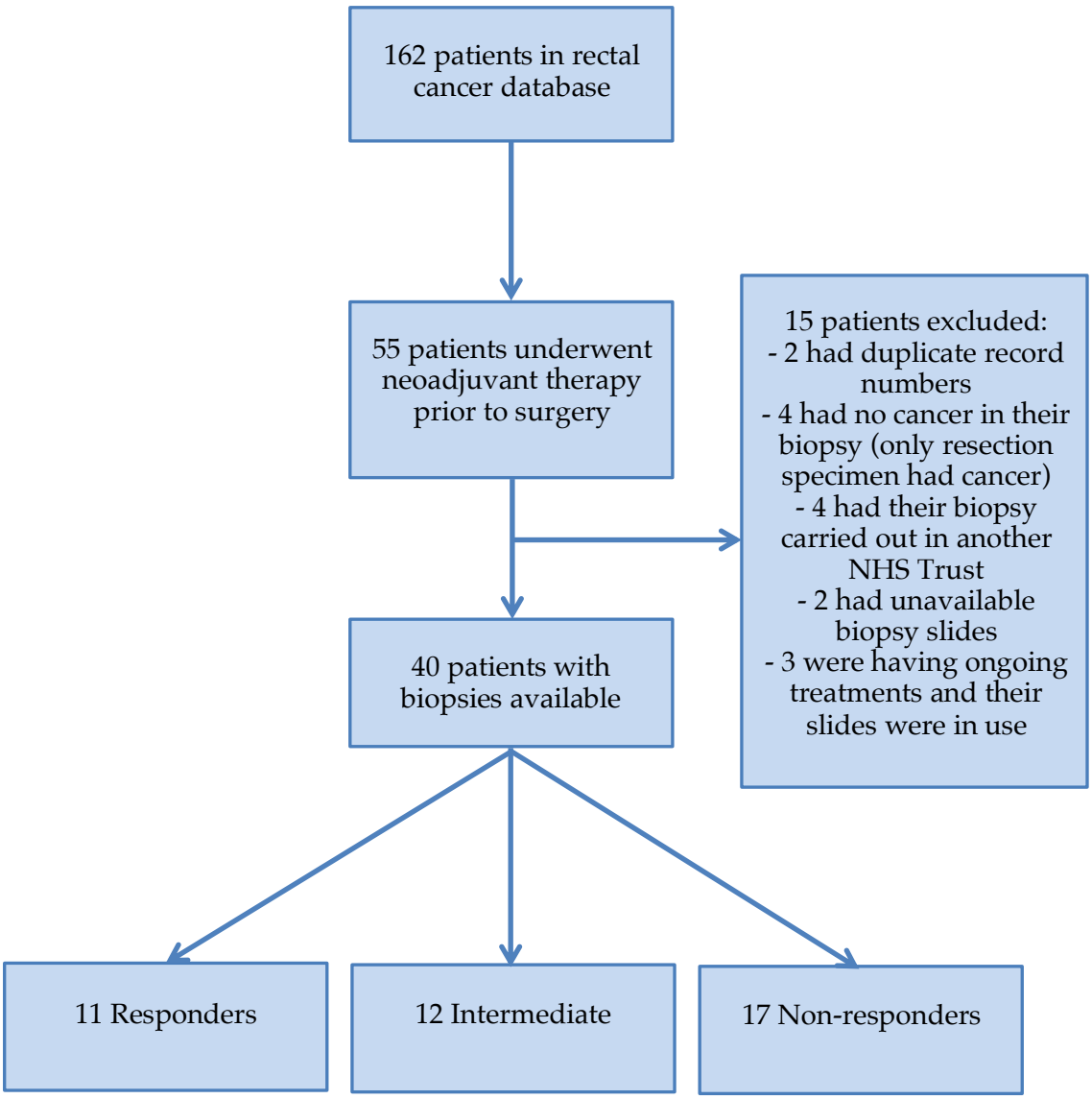


Table 5.2 Cohort of responders and non-responders

	Database number	Gender	Age	Ethnicity	Pre TNM staging	Pre UICC stage	NA therapy	Surgery	Histo TNM	Histo UICC	R	EMVI	TRG	Recurrence
RESPONDERS	182	Male	67	White British	T3N1	3	SCRT	AR	T3N0	2	0	-	4	None
	190	Male	74	White British	T4N1	3	SCRT	APER	T3N0	2	0	-	4	None
	264	Male	74	White British	T3N1	3	LCRT	AR	T3N0	2	0	-	4	None
	271	Male	67	White British	T3N1	3	LCRT	APER	T3N0	2	0	-	2	Systemic
	316	Female	64	White British	T3N2	3	LCRT	AR	T3N0	2	1	+	4	None
	352	Male	68	Bangladeshi	T3N1	3	SCRT	AR	T3N0	2	0	-	4	None
	385	Female	29	Bangladeshi	T3N0	2	LCRT	AR	T0N0	0	0	-	1	None
	519	Male	73	White British	T2N1	3	SCRT	AR	T2N0	1	0	-	4	None
	545	Female	63	White British	T3N2	3	LCRT	APER	T0N0	0	0	-	1	None
	629	Male	55	Bangladeshi	T3N2	3	LCRT	APER	T2N0	1	0	-	4	None
	710	Male	63	White British	T3N2	3	LCRT	AR	T1N0	1	0	-	3	None
NON-RESPONDERS	58	Female	63	White British	T2N0	1	SCRT	APER	T3N1	3	0	+	4	None
	76	Female	70	White British	T3N0	2	SCRT	AR	T3N0	2	0	-	4	None
	105	Male	71	Black Caribbean	T3N0	2	SCRT	APER	T3N0	2	0	-	4	Local
	110	Male	61	White British	T3N0	2	SCRT	AR	T3N2	3	1	+	4	None
	125	Male	84	Unknown	T3N0	2	SCRT	APER	T3N2	3	1	+	4	Local
	126	Female	68	White British	T3N1	3	SCRT	HP	T3N2	3	0	-	4	Systemic
	141	Male	78	White British	T3N0	2	SCRT	APER	T3N1	3	1	-	4	None
	175	Female	76	White British	T3N1	3	SCRT	APER	T3N1	2	1	-	4	None
	178	Male	70	White British	T3N1	3	SCRT	APER	T3N1	3	0	-	4	None
	240	Male	61	Indian	T3N1	3	SCRT	APER	T3N2	3	0	+	4	None
	284	Male	37	Bangladeshi	T2N0	1	LCRT	AR	T3N2	3	0	-	4	Systemic
	321	Female	72	White British	T3N1	3	SCRT	HP	T4N2	3	0	+	4	None
	374	Female	39	Black Caribbean	T4N2	3	LCRT	HP	T3N2	3	2	+	4	Local/systemic
	387	Male	80	Bangladeshi	T3N1	3	SCRT	HP	T4N1	3	0	+	4	Local/systemic
	434	Male	80	White British	T3N0	2	SCRT	APER	T3N0	2	0	-	4	None
	608	Male	68	White & Asian	T2N0	1	LCRT	Local	T2N0	1	0	-	4	None
	637	Female	73	Black Caribbean	T3N1	3	SCRT	AR	T3N2	3	0	+	4	Systemic

NA: neoadjuvant, histo: histological; R represents resection margins with R0: complete resection; EMVI: extra-mural venous invasion; SCRT: short-course radiotherapy; LCRT: long-course chemoradiotherapy; APER: abdomino-perineal excision of rectum; AR: anterior resection; HP: Hartmann's procedure (anterior resection with end colostomy); Local: local excision; UICC: Union for International Cancer Control; TRG: tumour regression grade.

5.4.2 Baseline data

The baseline characteristics of the responder and non-responder groups are compared in Table 5.3. The only significant difference pre-treatment was that more of the non-responders were node negative. In a similar way to the overall cohort, the majority of included patients were male (64.3%), underwent anterior resection (53.5%) and were White British (60.7%); the median age was 68 years.

Table 5.3 Baseline Characteristics IQR: interquartile range; SCRT: short-course radiotherapy; LCRT: long-course chemoradiotherapy; APER: abdomino-perineal excision of rectum; AR: anterior resection; EMVI: extra-mural venous invasion.

	Responders Number	Responders Percentage	Non-responders Number	Non-responders Percentage	p-value
Gender • Male • Female	 8 3	 72.7 27.3	 10 7	 58.8 41.2	0.689
Median age (IQR)	67 (63 – 73)	-	70 (62 - 77)	-	0.220
Ethnicity • White British • Other • Unknown	 8 3 0	 72.7 27.3 0	 9 7 1	 52.9 41.2 5.9	0.665
Pre UICC stage • 1 • 2 • 3	 0 1 10	 0 9.09 90.9	 3 6 8	 17.6 35.3 47.1	0.077
Pre treatment node status • Negative • Positive	 1 10	 9.09 90.9	 9 8	 52.9 47.1	0.041*
Neoadjuvant therapy • SCRT • LCRT	 4 7	 36.4 63.6	 14 3	 82.4 17.7	0.020*
Surgery • APER • AR or other	 4 7	 36.4 63.6	 8 9	 47.1 52.9	0.705
Histological stage • 0 • 1 • 2 • 3	 2 3 6 0	 18.2 27.3 54.5 0	 0 1 4 12	 0 5.9 23.5 70.6	<0.001*
R status • R0 • R1 or R2	 10 1	 90.9 9.09	 12 5	 70.6 29.4	0.355
EMVI • No • Yes	 10 1	 90.9 9.09	 10 7	 58.8 41.2	0.099
Recurrence • None • Recurrence	 10 1	 90.9 9.09	 10 7	 58.8 41.2	0.099

5.4.3 Results for miRNA extraction

The method described above for miRNA extraction was used for all samples. Satisfactory quantities of RNA were extracted and all samples achieved the 260/280 ratio of >1.6 required for good quality array. Table 5.4 shows the results for RNA extraction, 260/280 ratio and 260/230 ratio.

Table 5.4 Results for miRNA extraction

Responders n = 11			
Number	ng/µl	260/280	260/230
182	197.7	1.91	1.94
190	334.5	1.88	1.65
264	64.4	1.95	1.61
271	74.1	1.79	2.00
316	199.6	1.93	1.90
352	107.9	1.63	0.95
385	55.5	1.83	1.63
519	180.1	1.88	1.68
545	437.1	1.97	1.92
629	142.7	1.72	1.17
701	64.3	1.97	1.97

Non-responders n = 17			
Number	ng/µl	260/280	260/230
58	775.2	1.93	1.86
76	63.8	2.02	2.01
105	74.5	1.88	1.86
110	102.5	1.91	1.80
125	92.3	1.87	1.64
126	563.8	1.93	2.10
141	103.9	1.77	1.27
175	47.2	1.91	1.59
178	66.8	1.93	1.95
240	69.5	1.89	1.97
284	116.6	1.81	1.31
321	100.0	1.84	1.49
374	55.6	1.78	1.27
387	80.6	1.83	1.44
434	270.0	1.85	1.82
608	63.7	1.84	1.35
637	199.3	1.96	1.83

5.4.4 MicroRNA array results

The microRNA array contained 3100 capture probes covering 94% of human microRNAs in miRBase version 19.0[83]. For each microarray slide, Exiqon calculate the threshold of detection as 1.2 times the 25th percentile of the overall signal intensity of the slide. A total of 1241 probes were discarded by this filtering procedure due to having intensities above threshold in less than 20% (or 2) of the samples.

The array results showed that thirteen microRNAs were differentially expressed between the responder and non-responder groups. Table 5.5 shows the twenty microRNAs with the lowest p-value. To facilitate visualisation of the differential expression between responders and non-responders, $-\log^{10}$ p-values were plotted against $\log^{2}$ fold change values, see Figure 5.3. Eleven microRNAs were significantly decreased and two were significantly increased in responders vs. non-responders ($p<0.05$). A heatmap showing the normalised expression values of the top fifty microRNAs with the highest standard deviation is shown in Figure 5.4.

The results shown in Table 5.5 and Figure 5.3 show unadjusted p-values. With adjustment for multiple testing, with the Benjamini-Hochberg correction, none of the microRNAs had a p-value <0.05.

MicroRNA	Fold change	p-value
hsa-miR-574-3p *	0.691	0.002
hsa-miR-4539 *	0.566	0.003
hsa-miR-4303 *	0.826	0.016
hsa-miR-212-3p *	0.856	0.019
hsa-miR-1914-5p *	0.788	0.019
hsa-miR-4686 *	0.841	0.020
hsa-miR-505-5p *	1.151	0.026
hsa-let-7b-3p *	0.877	0.032
hsa-miR-197-3p *	0.795	0.037
hsa-miR-4425	0.768	0.040
hsa-miR-1255a	1.159	0.044
hsa-miR-92b-3p *	0.843	0.047
hsa-miR-330-3p *	0.724	0.048
hsa-miR-3124-3p	0.780	0.051
hsa-miR-23c *	0.857	0.055
hsa-miR-215-5p*	0.854	0.058
hsa-miR-4491	0.609	0.062
hsa-miR-4538	0.768	0.064
hsa-miR-483-5p *	0.779	0.065
hsa-miR-548t-5p	1.162	0.068

Table 5.5 MicroRNAs with lowest p-values in array results. MicroRNAs marked with * are those included in the technical validation.

Figure 5.3 Volcano plot showing results for all microRNAs identified by the array. The blue lines represent p=0.05 and p=0.01 and points above these lines represent statistically significant results.

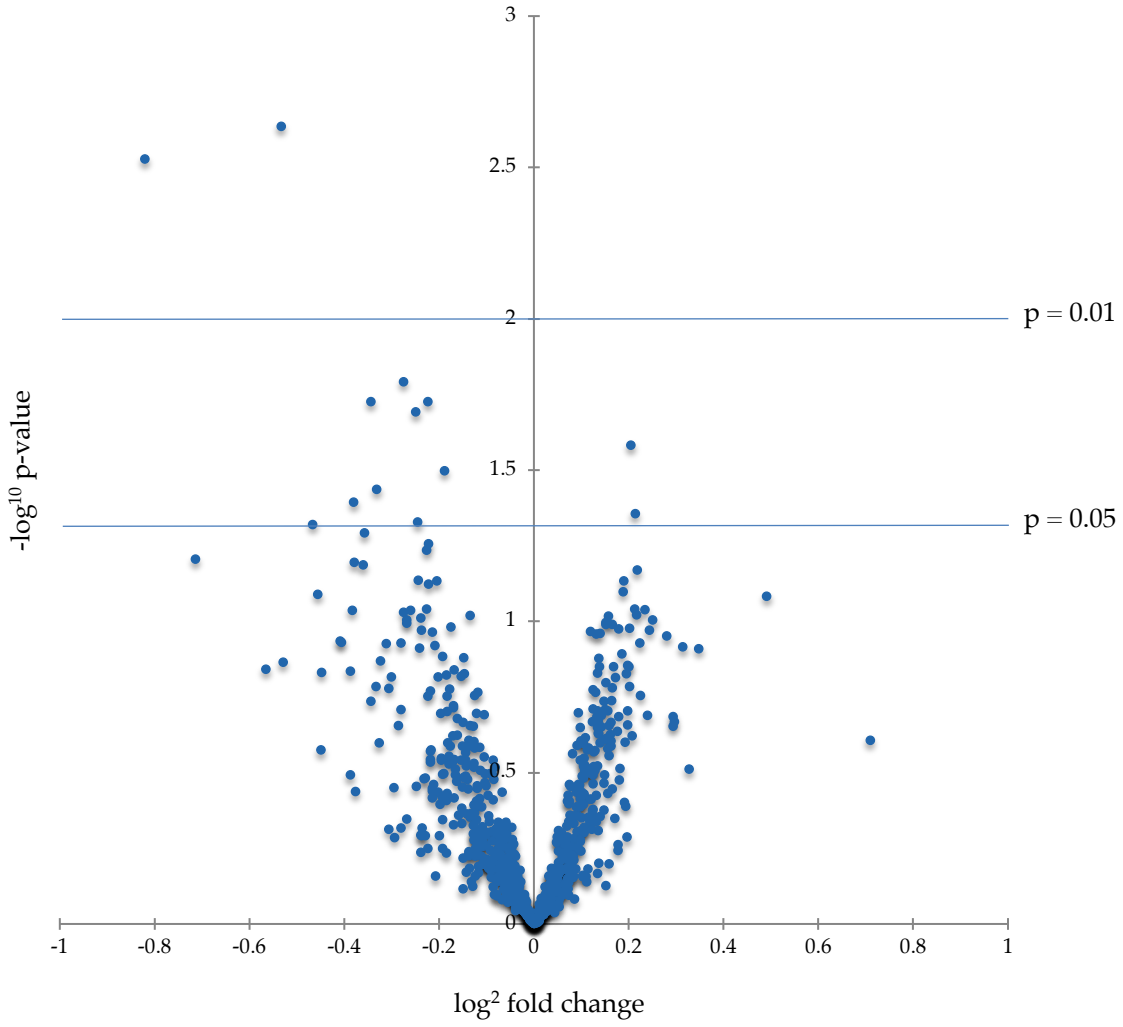
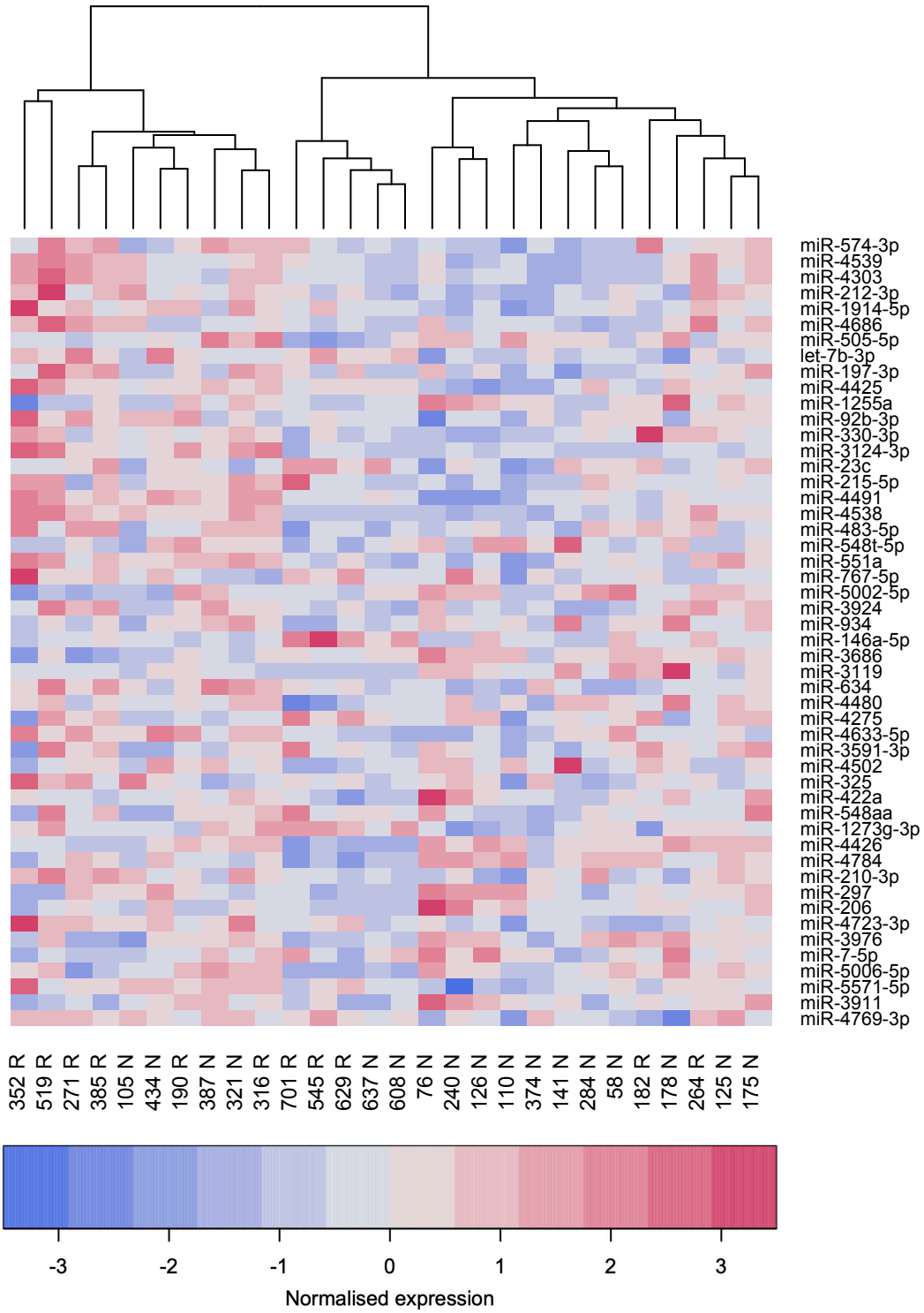


Figure 5.4 Heatmap of normalised expression levels in the top fifty microRNAs with the highest standard deviation



5.4.5 Results of technical validation: Responders vs. Non-responders

MicroRNA real-time qPCR analysis was used to technically validate the findings of the microRNA array results. The methods section gives details of the microRNAs chosen for validation. These were selected based on array results and/or relevant previous studies from the literature (see section 5.3.6 of methods for references). Normalisation for the qPCR was based on the average of the assays detected in all the samples as this has been shown to be an acceptable method for studies involving numerous assays[52].

During qPCR technical validation of the array results, the selected twenty-six microRNAs were measured in the 11 responder and 17 non-responder samples. Three of these were significantly downregulated in responders (miR-92b-3p, p=0.008; miR-197-3p, p=0.017; miR-4303, p=0.024). The remaining twenty-three microRNAs were not significantly different between the two groups. Five of the microRNAs were detected in too few samples to allow comparison between the two groups (miR-23c, miR-422a, miR-767-5p, miR-495-5p and miR-4686). Table 5.6 and 5.7 show the qPCR results for fold change and p-values. Figure 5.5 shows a volcano plot created to facilitate easy visualisation of the differential expression between responders and non-responders. Figure 5.6 shows results for the three microRNAs with significantly different expression between the two groups. A heatmap was constructed showing normalised expression levels for the twenty-six microRNAs included in validation for all samples, the results of this are shown in Figure 5.7.

Table 5.6 Normalised qPCR results for responders and non-responders: Results for microRNAs upregulated in responders. miR-23c was detected in too few samples to calculate a p-value.

MicroRNA	Non-responder	Responder	Fold change	p-value
miR-215-5p	2.425	3.089	1.585	0.106
miR-192-5p	3.386	3.979	1.508	0.180
miR-483-5p	-5.174	-3.882	2.448	0.311
miR-4539	-4.895	-4.430	1.380	0.352
miR-210-3p	-0.390	-0.147	1.183	0.422
miR-146b-5p	-3.656	-3.325	1.258	0.535
miR-342-5p	-6.148	-5.911	1.179	0.619
miR-29c-3p	0.875	0.983	1.077	0.683
miR-330-3p	-5.474	-5.456	1.012	0.974
miR-23c	-9.727	-7.548	4.529	-

Table 5.7 Normalised qPCR results for responders and non-responders: Results for microRNAs downregulated in responders. miR-422a, miR-767-5p, miR-495-5p and miR-4686 were detected in too few samples to calculate p-values.

MicroRNA	Non-responder	Responder	Fold change	p-value
miR-92b-3p	-2.368	-3.324	0.515	0.008
miR-197-3p	-0.621	-1.315	0.618	0.017
miR-4303	-6.538	-8.251	0.305	0.024
miR-451a	2.323	1.099	0.428	0.067
miR-505-5p	-5.459	-6.210	0.594	0.092
miR-574-3p	0.636	0.225	0.753	0.207
miR-206	-7.431	-8.207	0.584	0.242
let-7b-3p	-2.362	-2.779	0.749	0.278
miR-221-5p	-5.282	-5.942	0.633	0.414
miR-1914-5p	-4.348	-5.118	0.586	0.776
miR-212-3p	-4.660	-4.731	0.952	0.887
miR-29b-3p	0.951	0.941	0.993	0.970
miR-422a	-8.058	-8.101	0.970	-
miR-495-5p	-6.523	-8.433	0.266	-
miR-4686	-7.017	-9.525	0.176	-
miR-767-5p	-7.614	-	-	-

Figure 5.5 Volcano plot for responder and non-responder samples for the twenty-six microRNAs included in validation. The blue lines represent p=0.05 and p=0.01 and points above these lines represent statistically significant results.

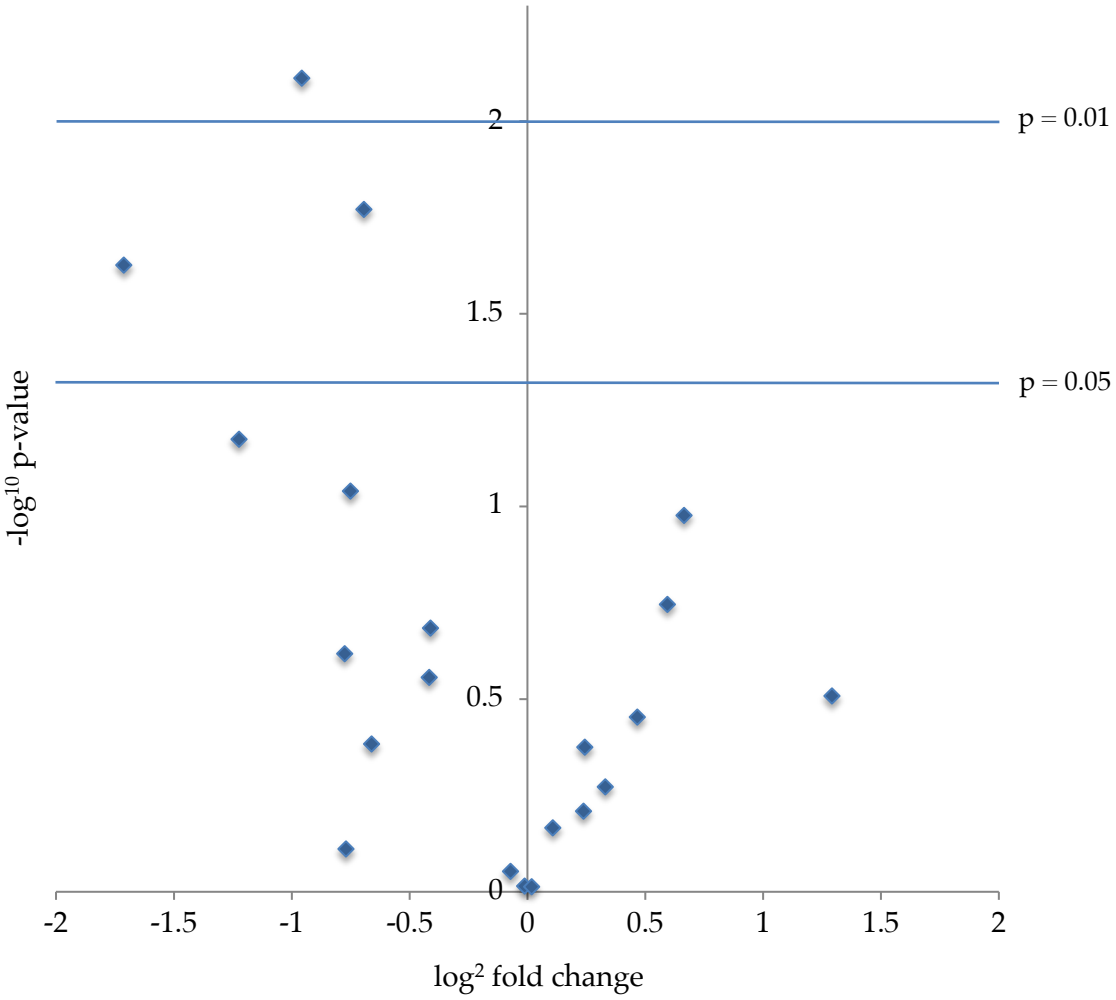
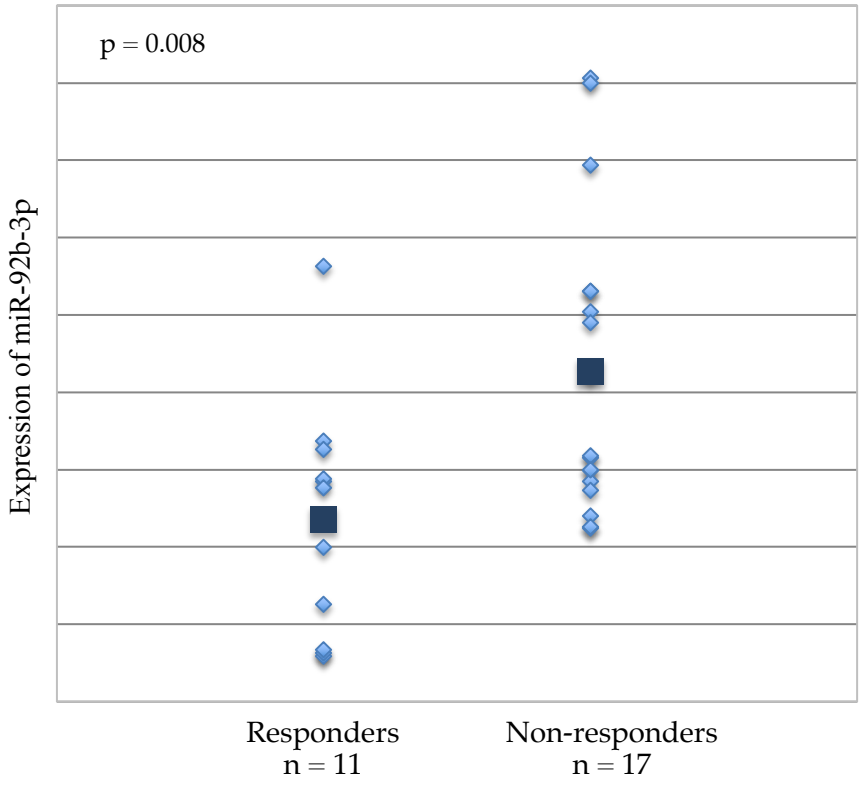
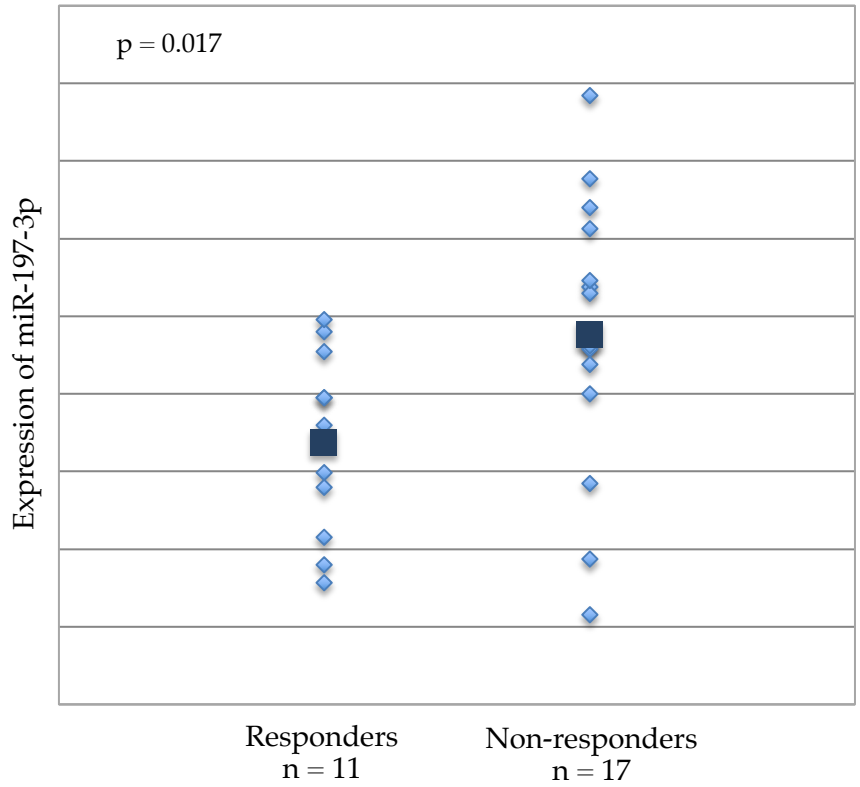
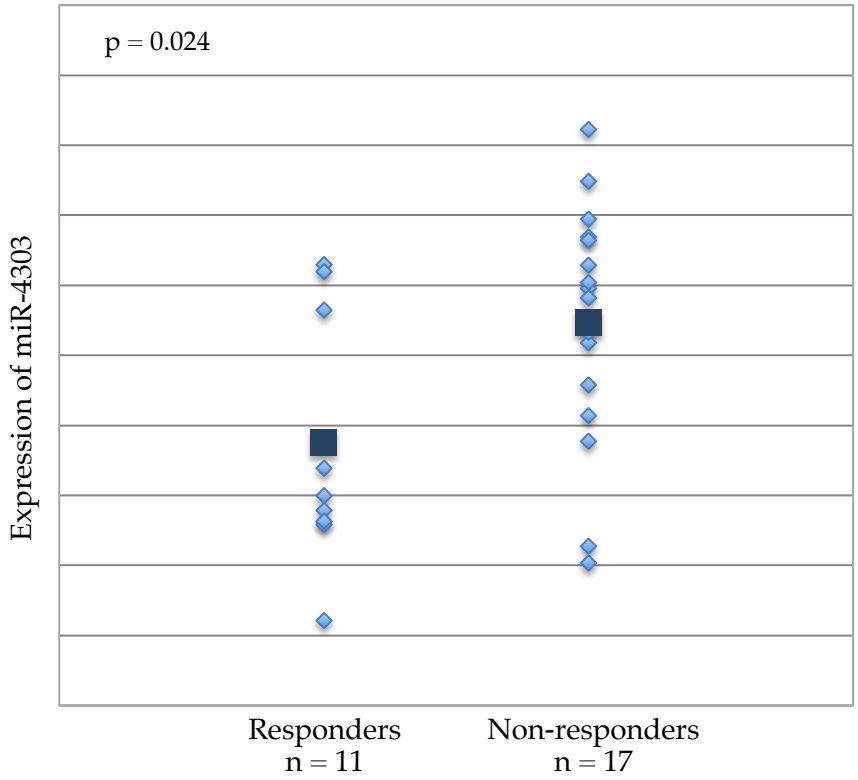


Figure 5.6 Expression levels of three microRNAs with significantly different expression between responders and non-responders. Large dark blue square represents mean expression.







ROC curves, comparing the three microRNAs with significantly different expression between responders and non-responders, are shown in Figure 5.8. A ROC curve was also constructed for the combination of the three microRNAs into one test and this is also included in Figure 5.8.

The highest AUC for an individual microRNA was 0.775 (95% CI 0.599 – 0.952) for miR-197-3p, this produced an optimal cut-off of 1.012 which showed 82% sensitivity, 73% specificity and 79% accuracy for detection of non-responders. The combination of the three microRNAs showed an even higher AUC of 0.913 (95% CI 0.797 – 1.000). Table 5.8 shows the optimal cut-off points derived from each ROC curve and the sensitivity, specificity, PPV, NPV and accuracy for each measure.

Using binary logistic regression with miR-92b-3p, miR-197-3p and miR-4303 combined, correctly predicted 70% of responders and 93.8% of non-responders, with overall 84.6% of samples correctly predicted using these three microRNAs.

Figure 5.7 Heatmap of normalised expression levels for the twenty-six microRNAs included in the validation

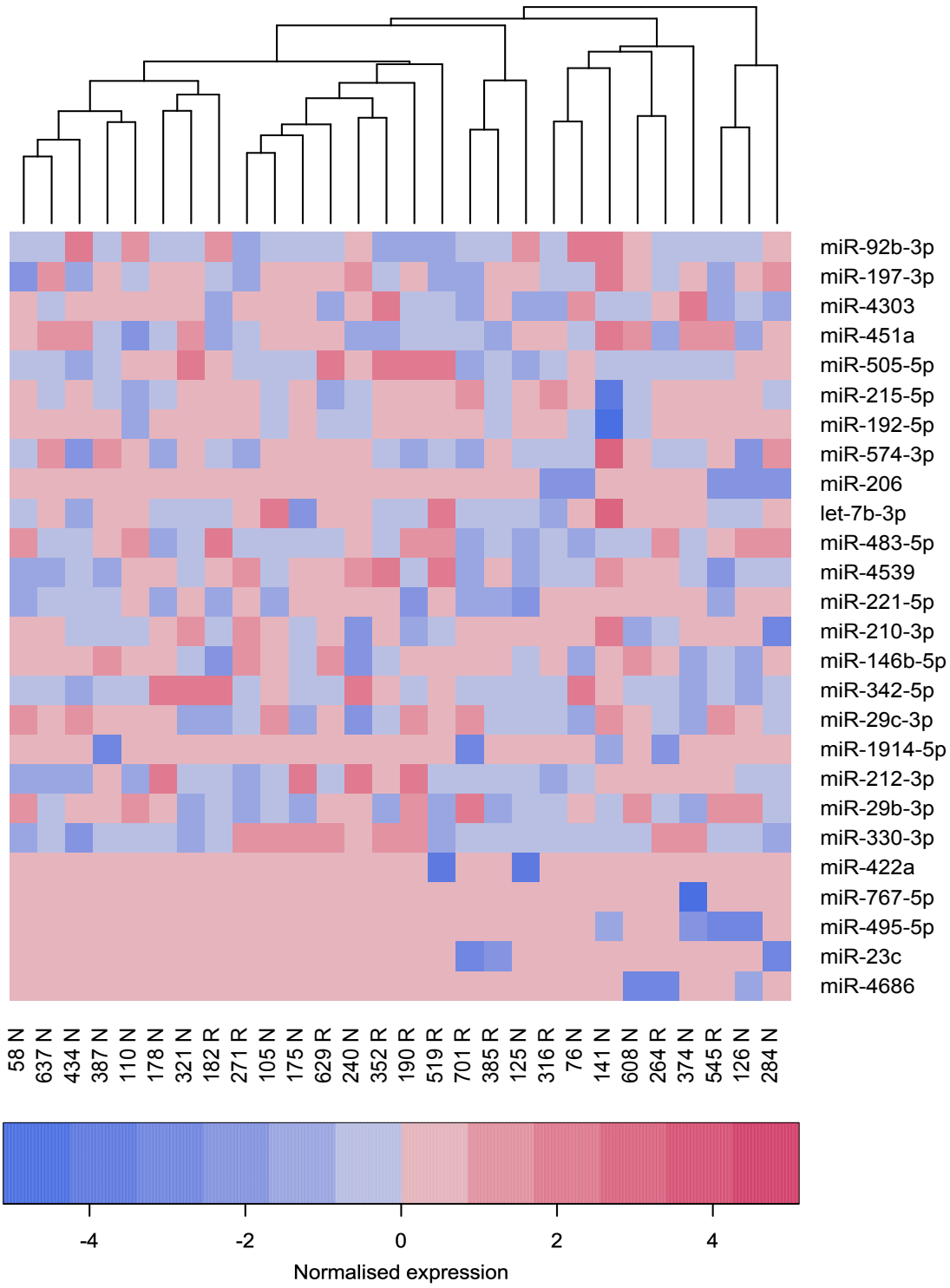


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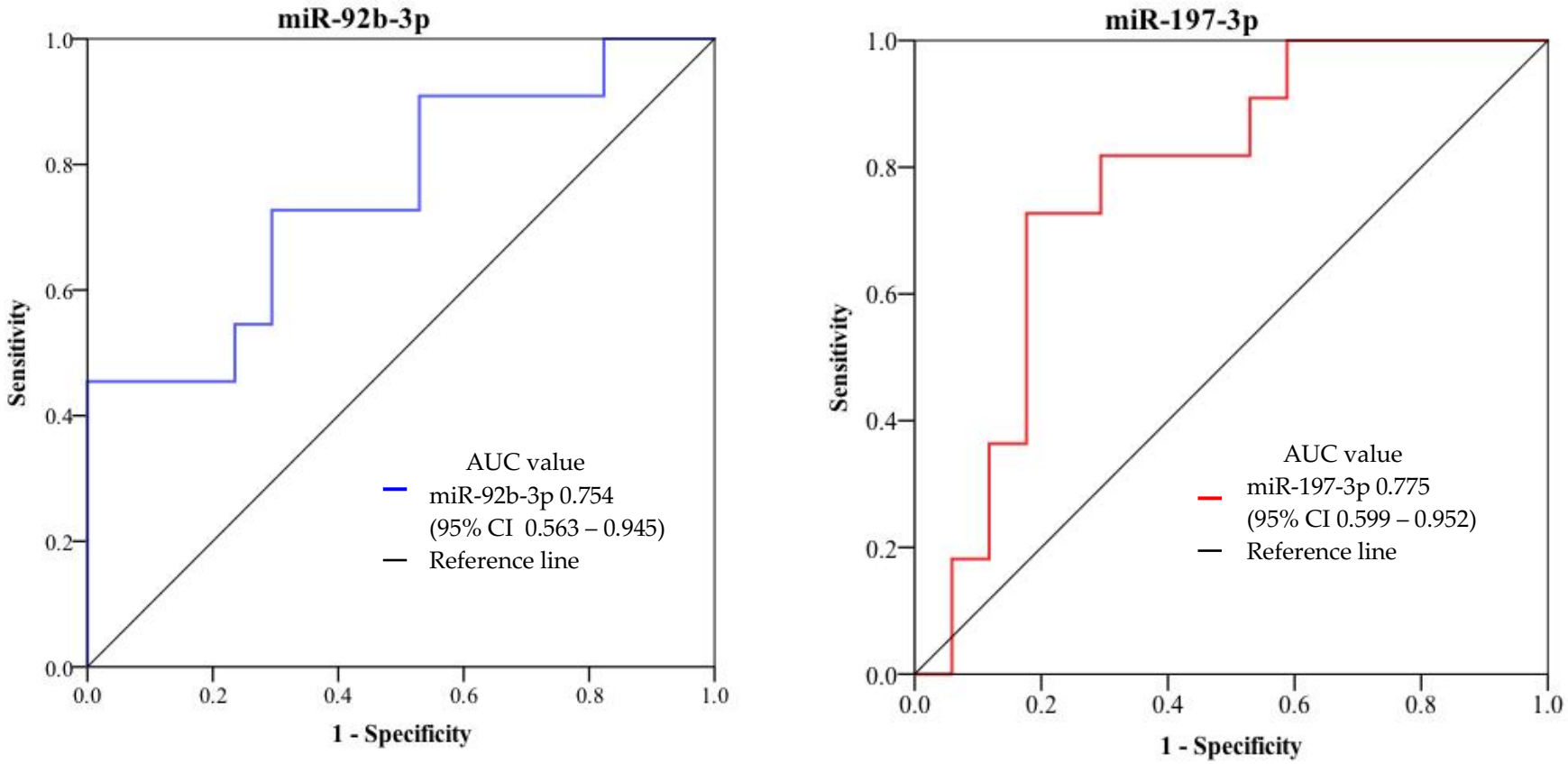
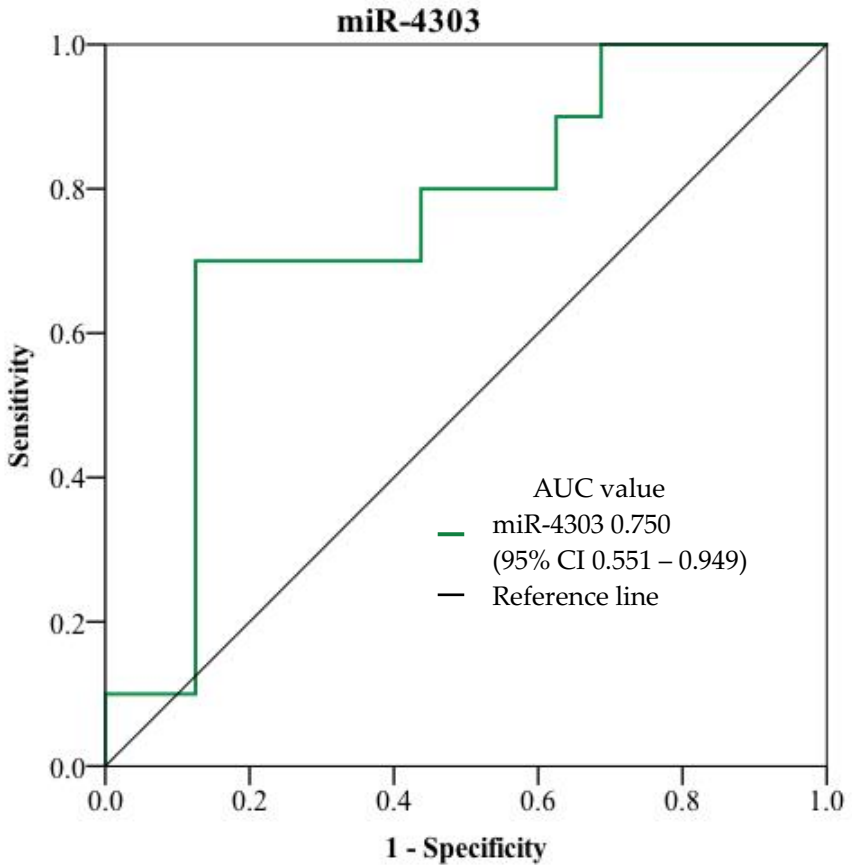
Figure 5.8 ROC curves demonstrating the ability of the microRNAs to act as diagnostic tests to differentiate between responders and non-responders. ROC: receiver-operating characteristic; AUC: area under the curve; 95% CI: 95% confidence intervals.




miR-4303
Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
AUC value
miR-4303 0.750
(95% CI 0.551 – 0.949)
Reference line

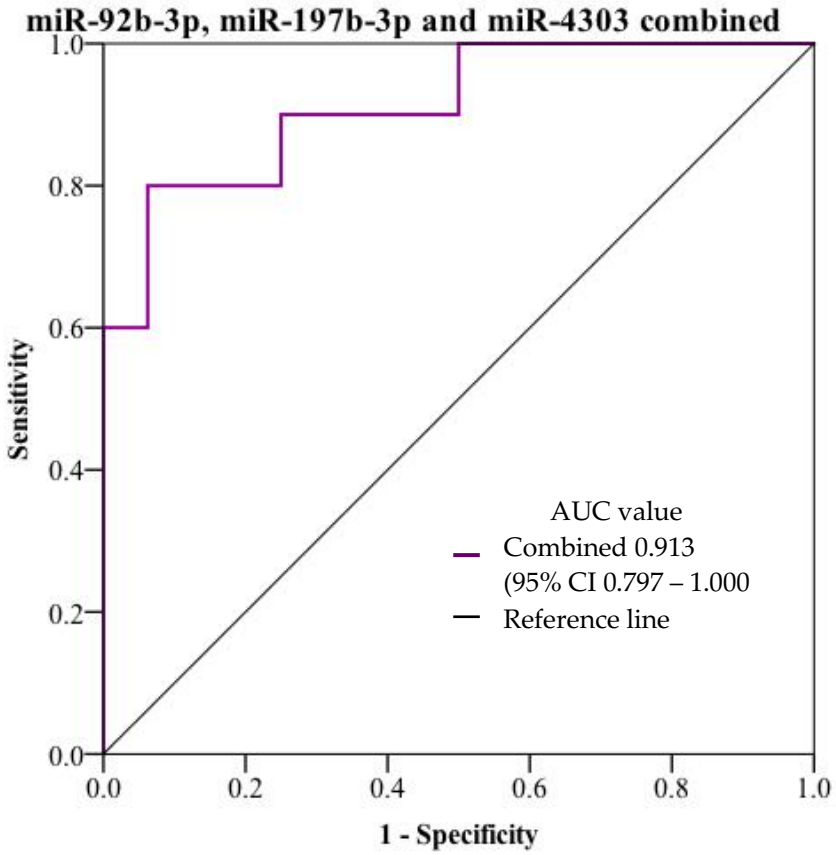

miR-92b-3p, miR-197b-3p and miR-4303 combined
Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
AUC value
Combined 0.913
(95% CI 0.797 – 1.000
Reference line

Table 5.8 Sensitivity, specificity, PPV, NPV and accuracy of the three microRNAs, and the combination of all three, when used as diagnostic tests for differentiating non-responders from responders. Values in brackets are 95% confidence intervals. ROC: Receiver-operating characteristic; PPV: positive predictive value; NPV: negative predictive value.

	Cut-off derived from ROC curve	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %	Accuracy %
miR-92b-3p	-3.441	100 (84 – 100)	45 (17 – 77)	74 (52 – 90)	100 (55 – 100)	79 (59 – 92)
miR-197-3p	-1.012	82 (57 – 96)	73 (39 – 94)	82 (57 – 96)	73 (39 – 94)	79 (59 – 92)
miR-4303	-8.236	88 (62 – 98)	80 (44 – 97)	88 (62 – 98)	80 (44 – 97)	81 (61 – 93)
Combined	0.556	94 (70 – 100)	80 (44 – 97)	88 (64 – 99)	89 (52 – 100)	88 (70 – 98)

MicroRNA target prediction databases miRWalk database version 3.0[82] and miRDB[83] were used to identify possible target genes for the three microRNAs showing significantly different expression between responders and non-responders. The list of genes was then reviewed with reference to the relevant literature and a list of putative targets derived. These are shown in Table 5.9. The target genes identified are examined in more detail in the discussion section below.

Table 5.9 Putative target genes predicted using miRWalk, miRDB and with reference to literature. FBXW7: F-box and WD-40 domain protein 7; NLK: Nemo-like Kinase; DKK3: Dickkopf-3 gene; BCL2L11: Bcl-2-like protein 11; PER2: period circadian regulator 2; PTEN: Phosphatase and tensin homolog; SMAD3: Mothers against decapentaplegic homolog 3; ITGA6: Integrin α6; DAB2IP: Disabled homolog 2-interacting protein; CKS1B: cyclin-dependent kinase CDC28 protein kinase regulatory subunit 1B; STAT3: Signal transducer and activator of transcription 3; Bcl-2: B-cell lymphoma 2; MCL1: Myeloid cell Leukemia 1.

MicroRNA	MicroRNA sequence	Putative targets
miR-92b-3p	UAUUGCACUCGUCCCGGCCUCC	FBXW7[84], NLK[85], DKK3[86], BCL2L11[87], PER2[88], PTEN[89], SMAD3[90], ITGA6[91], Rab23[92], DAB2IP[93]
miR-197-3p	UUCACCACCUUCUCCACCCAGC	p53[94], NLK[95], CKS1B[96], STAT3[96], Bcl-2[96], MCL1[97]
miR-4303	UUCUGAGCUGAGGACAG	-

5.4.6 Results of technical validation: Hypoxia samples

Along with the 28 responder and non-responder samples, the twenty-six microRNAs selected for technical validation were also measured in samples from three rectal cancer cell lines (HT55, SW837 and VACO4s), grown under three different oxygen conditions (0.2% oxygen, 1% oxygen and 20.9% oxygen) for 48 hours. The only microRNA which showed a significant difference in expression between the oxygen tensions was miR-210-3p (ANOVA between oxygen tensions p = 0.019; T-test for

normoxia to 0.2% oxygen p = 0.002). There was no significant difference identified between oxygen tensions for any of the other microRNAs including miR-92b-3p, miR-197-3p and miR-4303.

5.5 Discussion

The management of patients with rectal cancer has improved considerably in recent years, partly due to improvements in staging and the use of neoadjuvant therapy[98]. Despite this, the individual tailoring of therapy remains one of the major challenges facing clinicians who treat rectal cancer[98]. New technology has the potential to identify prognostic molecular biomarkers that could enable this goal. A lack of response to neoadjuvant therapy is associated with poor prognosis in terms of poorer disease-free survival[99] and tumour regression grade has been shown to be a better prognostic factor than downstaging[100]. 5-year survival is over 90% for patients with a histological complete response (TRG 1) following neoadjuvant therapy and these patients have a 3.3-fold advantage in overall survival compared with incomplete responders[101].

A biomarker for the prediction of response to neoadjuvant CRT would allow tailoring of therapy on an individualised level, rationalising the use of CRT, which is known to have a negative impact on functional outcome[1]. As outlined in the introduction above, it could also facilitate avoidance of surgery altogether in those patients predicted to respond well to CRT who opted to follow an intensified regime aiming from the outset to avoid surgery if possible. Even without a predictive biomarker stratifying use, an intensified neoadjuvant CRT regime has been shown to achieve up to 49% early complete clinical response of the tumour[102] indicating that a sizeable group of patients could potentially benefit from this strategy.

This exploratory study has confirmed that extraction of microRNAs from pre-treatment rectal biopsy tissue blocks is feasible and can yield sufficient RNA to facilitate array profiling. The results have identified that expression of microRNAs potentially differs between responders and non-responders to neoadjuvant CRT showing that microRNAs have potential as predictive biomarkers. There is some overlap between the methodology used in this study and previous similar studies but this study has identified further microRNA targets, which could form the basis for future research to validate and build on these initial findings.

For two of the microRNAs identified, miR-92b-3p and miR-197-3p, there are established links to response to chemo/radiotherapy in several cancers and studies from the literature potentially explain the mechanisms of chemoresistance which could be linked to altered expression[84,96,103-109].

miR-4303 was identified in 2009, based on deep sequencing[110] and is predicted to be a microRNA based on its sequence but it has not yet been functionally validated[111]. It is relatively unknown in the literature and the only previously reported association with cancer is that expression was found to be downregulated in gastric cancer[112].

miR-92b-3p is known to be an oncogenic microRNA[103] with increased expression previously identified in several cancer types, including colorectal cancer[84], glioblastoma[103], lung cancer[104], sarcoma[113], nasopharyngeal carcinoma[90], oral squamous cell carcinoma[114], oesophageal squamous cell carcinoma[91], hepatocellular carcinoma[115], cholangiocarcinoma[87], bladder cancer[93] and osteosarcoma[88]. MicroRNA array analysis has shown that is it upregulated in metastatic colorectal cancer tissues[84] and further studies have shown that miR-92b-3p promotes proliferation, migration and invasion in colorectal cancer[84]. It has been shown to play a similar role in the proliferation of glioblastomas[103]. miR-92b functions as an oncogene in non small cell lung cancer regulating cell growth and is also upregulated in cisplatin chemotherapy resistant cells[104]. As well as a role in proliferation and resistance to therapy, it also has a potential role in recurrent disease, it was highly expressed in patients who developed recurrence following curative surgery and adjuvant chemotherapy for gastric cancer[116].

Several putative target genes were identified for miR-92b-3p. F-box and WD-40 domain protein 7 (FBXW7) is a tumour suppressor gene associated with resistance to chemotherapy[105]. miR-92b-3p has been shown to inhibit FBXW7 in vitro and thereby promote colorectal cancer development[84]. Nemo-like Kinase (NLK) is also a tumour suppressor gene, identified to play a role in colorectal cancer[117]. NLK is a direct target of miR-92b-3p and elevated levels affect glioma proliferation through NLKs effects on the Wnt/beta-catenin signalling[85]. Dickkopf-3 gene (DKK3) is a further target gene of miR-92b, which acts as an antagonist of the same Wnt/beta-catenin signalling pathway[118]. miR-92b inhibits the expression of DKK3 and can thereby regulate cell proliferation and apoptosis in gliomas[86].

Bcl-2-like protein 11 (BCL2L11) is part of the Bcl-2 (B-cell lymphoma 2) family of proteins, and is a downstream gene of the Wnt/beta-catenin signalling pathway[119]. The period circadian regulator 2 (PER2) gene plays a potential role in tumor suppression through regulation of DNA-damage-responsive pathways[120], it is also partly regulated by beta-catenin[121]. BCL2L11 and PER2 have both previously been identified as potential targets of miR-92b in cholangiocarcinoma[87].

MicroRNA miR-197 is known to function as an oncoMIR, playing several key roles in cancer progression including effects on proliferation, differentiation, metastasis, invasion, apoptosis and drug resistance[106,107]. Altered expression has been identified in many types of cancer including colorectal cancer[108], lung cancer[94], breast cancer[122], ovarian cancer[95], hepatocellular carcinoma[123], thyroid cancer[124], pancreatic cancer[125] and osteosarcoma[126]. miR-197 was shown to be downregulated in colorectal cancer cell lines following 5-fluorouracil (5-FU) chemotherapy[109] and a further study by the same group showed that miR-197 mediated resistance to 5-FU via regulation of thymidylate synthase expression[108]. Similarly, miR-197 was significantly increased in ovarian cancer cells showing resistance to Taxol chemotherapy and expression of miR-197 promoted Taxol resistance, proliferation, and invasion[95]. In non-small cell lung cancer, miR-197 is downregulated in platinum chemotherapy-resistant specimens and regulates drug resistance and tumour progression[96]. miR-197-3p has been identified as a possible therapeutic target in thyroid cancer as overexpression promotes migration and invasion[124]. Similarly, miR-197 overexpression has been shown to promote invasion in pancreatic cancer[125] and bladder cancer[127].

As with miR-92b-3p, several putative target genes were identified for miR-197-3p. p53 is one of the best known tumour suppressor genes and is frequently mutated in human cancers[128]. In lung cancer cells, downregulation of miR-197 was shown to induce p53-dependent lung cancer cell apoptosis with down-modulation of miR-197 leading to significant upregulation of the p53 pathway[94]. Another tumour suppressor gene, NLK, which is also a direct target of miR-92b-3p (see above), is also downregulated by miR-197, promoting drug resistance in ovarian cancer cells[95].

As outlined in the introduction, hypoxia is known to play a key role in response to both chemotherapy and radiotherapy[14]. The samples from rectal cancer cell lines grown in normoxia and hypoxic conditions, showed significant differences in expression of miR-210-3p. The other microRNAs, including the three identified as linked to response to neoadjuvant therapy, did not show any difference in

expression between the different oxygen tensions, indicating that mechanisms other than hypoxia are probably more important for these microRNAs.

The finding that miR-210 is increased in hypoxia, is expected, as miR-210 is known to be a master hypoxamir, induced under hypoxic conditions[129] and was included in the validation set of microRNAs for this reason. miR-210 is upregulated in most solid tumours, high levels are linked to hypoxia and are associated with poor prognosis in terms of clinical outcomes[62]. When hypoxia is present in a tumour, hypoxia inducible factors (HIF) are activated[129]. These control cellular responses to hypoxia including metabolism, proliferation and invasion. miR-210 is a known target of both HIF1α and HIF2α[130]. miR-210 is specifically increased in hypoxic regions of colorectal cancers and has been suggested as a potential biomarker for hypoxia in these cancers[63].

There are an extensive number of studies attempting to identify predictive molecular biomarkers for response to neoadjuvant therapy in rectal cancer. As outlined in the introduction, few of the markers identified have subsequently been verified by external validation or independent studies. Biomarkers for routine clinical use would need to show accuracy and consistency, as well as applicability to relevant patient groups and, so far, none have achieved this sufficiently to be adopted. Establishment of the mechanistic links between biomarkers and tumour biology are crucial to their use and there are other considerations including cost-effectiveness[3]. It is likely that a single marker may not be able to meet these challenges and instead, a panel, or combination of molecular biomarkers could be used to stratify risk. A combination of three molecular biomarkers, c-MYC, PCNA and TIMP1 has been previously used in an attempt to predict outcome in rectal cancer, in order to improve predictive accuracy compared with single markers alone[131]. The same study went on to combine this molecular panel with MRI-detected extramural venous invasion, an imaging biomarker, and was able to improve categorisation of patients into prognostic groups ($p < 0.01$) [131].

Currently none of the predictive molecular markers used in rectal cancer are in routine clinical use. This contrasts with other cancers, in particular breast cancer, where biomarkers play an essential role, both in diagnosis and in predicting outcome[132]. Multigene panels are used for breast cancer patients in clinical practice, to provide prognostic information about disease free survival and assist decision-making about chemotherapy[132].

The comparison of baseline characteristics between the responder and non-responder groups show that they are well matched with the only pre-treatment difference being a higher proportion of responders having nodal involvement. There was no difference in pre-treatment staging but as would be expected, histological stage was significantly lower in responders to neoadjuvant therapy.

The two groups were not exactly matched in terms of the neoadjuvant therapy received, more of the non-responders had short course radiotherapy, while more of the responders had long course chemoradiotherapy. This lack of matching between the two groups is one of the limitations of the study. It would also have been preferable to have defined response purely in terms of tissue regression grade with only those with TRG 1 or even TRG 1 or 2 in the responder group. Relying on downstaging as a marker of response, inherently relies on the accuracy of MRI for staging rectal cancer pre-treatment, the limitations of which have been discussed in a previous chapter. The decision to include downstaging as well as TRG in defining response was down to limitations on the number of samples available.

The sample size was limited by several factors. The study could only include patients within the Colorectal Cancer Tissue Bank database as these patients had given ethical approval, including for retrieval and use of their pathology blocks. There were 55 patients in the database that had neoadjuvant therapy for rectal cancer. A proportion of these were excluded (see Figure 5.2), firstly it was not possible to retrieve biopsy slides for all patients, as these were stored off-site for historical slides. As this unit is a tertiary centre some patients underwent their biopsy in a separate NHS Trust and further patients did not have cancer present in their biopsy slides. A few patients were having ongoing treatment and their pathology slides were in use and 2 patients had duplicate numbers in the database and unfortunately had to be excluded. This left 40 eligible patients. Of these, 12 patients were not clearly responders or non-responders, they had mixed results, for example, worsening of their stage from pre treatment staging to histological stage but TRG 2. This resulted in the overall sample of 28 patients and the smaller sample size limited subgroup analysis and may have lead to type II errors.

Further limitations of the study are that not all characteristics of the tumours were not known, including height of the tumour from the anal verge, one tumour related factor that can affect outcome[133]; however, this is unlikely to have had much impact, as the proportion undergoing abdominoperineal excision rectum (APER) was not

significantly different between the two groups. No independent validation group was used to verify the results and this is obviously still required, but again was limited by available numbers.

There are some limitations associated with the methodology of this type of study. The use of pre-treatment biopsies is required to ensure that the microRNAs have not been altered by therapy received, and because this is the tissue that would be used in the real world to measure any predictive biomarker which could be established. However, the biopsies taken are usually from the surface of the tumour, and marked heterogeneity within cancers may mean that these may not be representative[134], this is however, a limitation of all diagnostic methods using biopsies.

Further work to build on these results would need to start with validation in independent sample cohorts, ideally via collaboration with other clinical centres and laboratories using standardised methodologies. The validation set should ideally have larger numbers, with clear definition of response and non-response to achieve more homogenous groups. Work to try and achieve this is ongoing and unfortunately was not completed within the timescale of this thesis. The next step would be work to identify targets and further develop the possible mechanisms involved, this would be based on findings from previous studies outlined above and the list of putative targets identified. As described above, consideration would need to be given as to whether the microRNAs identified could be used alone, as a combination, or in combination with another modality, such as diffusion weighted MRI.

5.6 Conclusions

This exploratory study has confirmed that analysis of microRNA expression in pre-treatment rectal biopsies is feasible. Expression of microRNAs potentially differs between responders and non-responders to neoadjuvant CRT. MicroRNA targets therefore have potential as predictive biomarkers for response to neoadjuvant therapy in rectal cancer. This study has identified three possible targets for future validation studies. Further research into the development of molecular biomarkers will require a more sophisticated understanding of the mechanisms behind response and non-response to therapy.

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Chapter 6. Assessment of anal sphincter function following anterior resection using high-resolution anorectal manometry

6.1 Introduction

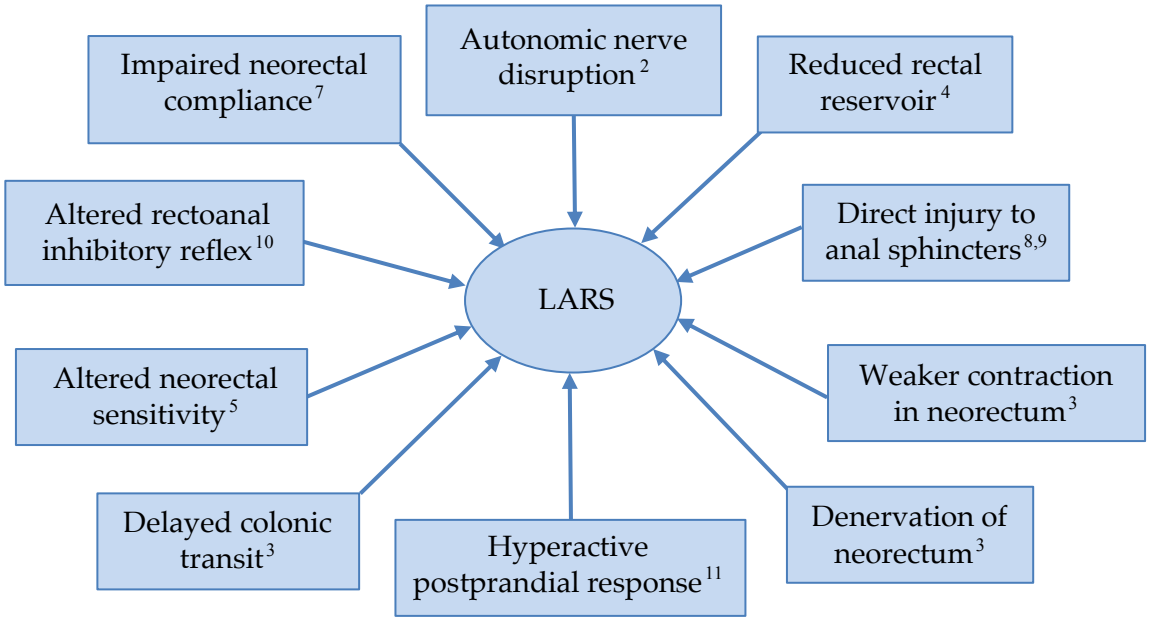
As detailed in chapter 3, low anterior resection syndrome (LARS) affects a large proportion of patients undergoing treatment for rectal cancer. Although risk factors have been identified, it is still unclear why the incidence varies even within the low and high-risk groups. The changes in anal sphincter structure and function that ultimately lead to LARS are not well understood and predicting which individual patients might develop problems remains a challenge. A better understanding of the pathophysiology underlying poor functional outcomes will be the only way to determine how we can improve the management of rectal cancer, with the overall treatment aim of preserving functioning sphincters.

LARS is a multifactorial condition with a contribution from several different mechanisms[1]. Figure 6.1 illustrates some of these contributory factors. Autonomic nerve disruption, with division of the inferior mesenteric ganglia and the hypogastric plexus during surgery, has long been thought to contribute to poor post-operative function[2], this is one of the few mechanisms believed to be partly preventable. During anterior resection, the rectum is replaced with the sigmoid or descending colon, this has thinner, weaker muscle, that does not contract as strongly and is relatively denervated compared with the rectum[3]. The neorectum also has altered motility, loss of the normal propagation of contraction and spastic waves have been demonstrated and these were shown to correlate with urgency and clustering[3]. Several studies have identified altered sensitivity following anterior resection[4-6], the reduction in reservoir size[4] and altered compliance[7] are thought to contribute to this.

It is clear that neoadjuvant chemoradiotherapy is a risk factor for LARS, but this effect has mostly been studied in combination with subsequent surgery and there is very little evidence about the effect of chemoradiotherapy alone on anorectal function. A better understanding of the effects of CRT separate to those of surgery would be useful to help minimise negative effects and also when counselling patients about the risks and benefits of different aspects of rectal cancer therapy. The effect of CRT can be difficult to study in rectal cancer patients as most of these patients undergo surgery following CRT. Increasingly some patients with a clinical

complete response to CRT are following a watch and wait protocol[12] rather than undergoing surgery. The functional outcomes of this newer treatment protocol are not known, and this patient group may be ideal for studying the effect of CRT alone on functional outcome[13].

Figure 6.1 Mechanisms contributing to LARS



Functional outcomes following treatment for rectal cancer have been poorly assessed in comparison to oncological outcomes[14]. As discussed in chapter 3, it is important that the routine assessment of functional outcome becomes normal practice, in order to identify symptomatic patients and refer them for treatment. However, it is equally important that we give consideration to the ways in which post treatment dysfunction can be minimised or prevented. This can only be achieved with improved understanding of pathophysiology.

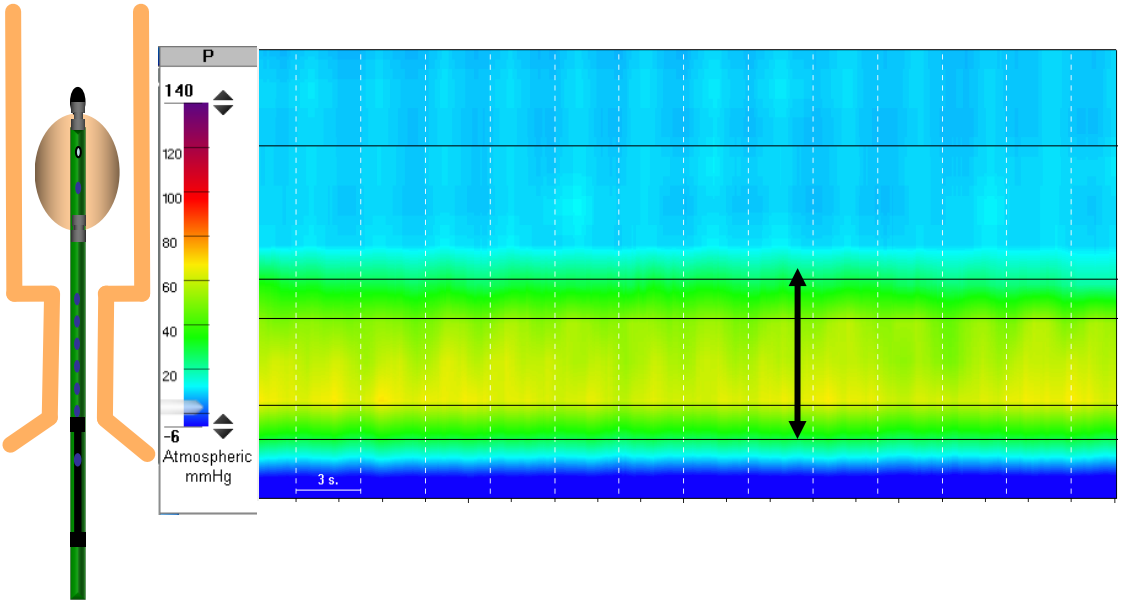
Anorectal manometry is the most commonly used investigation to assess anorectal function[15]. Studies have shown that anorectal manometry results can influence the diagnosis, management and outcome for patients with faecal incontinence and constipation[16,17]. It is therefore likely that it would be a very useful tool in the assessment of pre and post treatment function in rectal cancer patients. Despite the widespread use of manometry, there are acknowledged limitations of this assessment. The main difficulty in interpreting results is the variation in equipment and methodology between units[15]. This prohibits comparisons between different units and has led to lack of agreement about normal values and reference ranges[15].

Standard anorectal manometry utilises either a solid-state or water-perfused probe with transducers at 1cm intervals arranged radially[18]. This is then withdrawn 1cm at a time and repeated measurements taken. High-resolution manometry differs from traditional manometry as the catheter uses multiple transducers, closely spaced together, which simultaneously measure circumferential pressures[15], see Figure 6.2. The transducers are close enough together to allow intraluminal pressure to be measured as a continuum[19]. Software can then be used to convert the manometry readings into colour contour pressure topography plots, which allow appreciation of the anorectum as a functional unit and can also be used to show dynamic changes[19]. Examples of these colour contour plots are shown in Figure 6.3 and Figure 6.4.

Figure 6.2 UniTip HRAM catheter [20]

The initial use of high-resolution manometry was for investigation of oesophageal function and it has now been widely adopted for this assessment in preference to standard manometry[21]. In contrast to standard manometry there has been a focus on standardisation of techniques for anorectal assessment, with published definitions and protocols for the methods used[22]. The reference ranges for the normal population have also been provided by studies carried out on healthy volunteers[22,23]. High-resolution anorectal manometry (HRAM) using a standardised protocol is an objective and repeatable measure, ideal for use in rectal cancer patients with interacting factors affecting sphincter morphology and function.

Figure 6.3 HRAM colour contour display at rest. The pressure scale is shown to the left of the colour plot and the diagram to the far left demonstrates the manometry catheter position within the anal canal and rectum. On the colour plot, the functional anal canal length is displayed by the green band (measured by black arrow), low pressure (indicated by light blue band) is seen in the rectum.



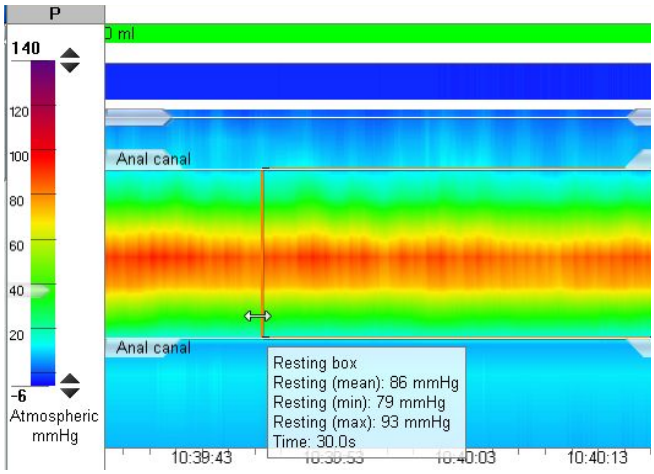
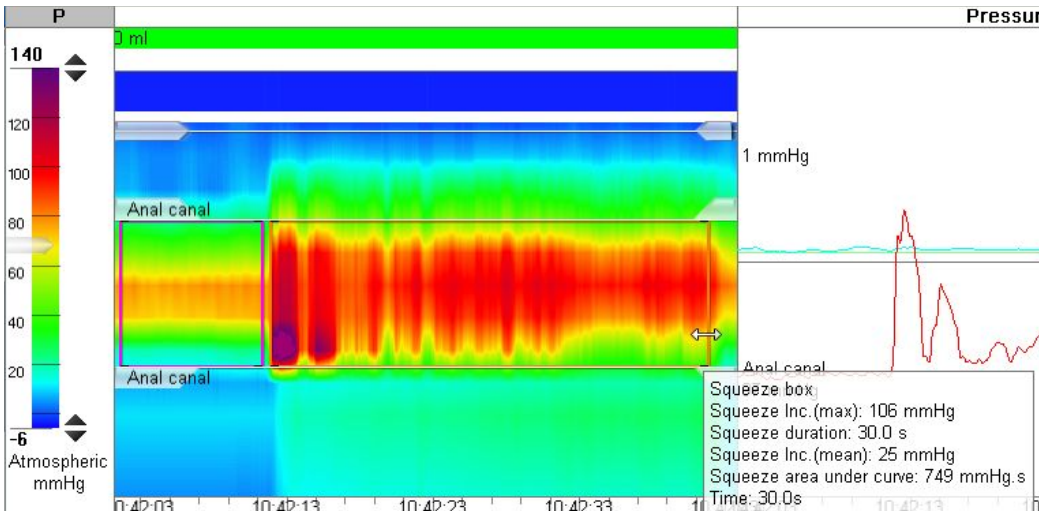


Figure 6.4 Representative HRAM colour contour displays at rest and during endurance squeeze manoeuvre. The red and yellow stripes in the anal canal during resting indicate the presence of normal slow wave activity. The rise in pressure in the anal canal during squeeze is due to the contraction of the external anal sphincter.



There is limited evidence about the link between LARS, or the component symptoms of LARS, and specific abnormalities on manometry testing[5]. This is partly due to a lack of studies considering this question. The studies that have been carried out have shown conflicting results, some show correlation between LARS and altered parameters including resting pressure, compliance and capacity measures[5] with others showing no difference in manometry results between patients with and without symptoms[24].

The advantages of HRAM over conventional manometry give it the potential to be a useful tool to better understand the physiological changes that underlie functional problems and determine whether treatment for rectal cancer can be improved in any way to better preserve function. It may also be a useful tool for the assessment of symptomatic patients. Although standard manometry has been studied in patients who have had an anterior resection, very few studies have used HRAM in rectal cancer patients, or compared findings at pre and post treatment timepoints. This provides an opportunity for an exploratory study to establish the use of HRAM as an investigative tool in these patients and build a basis for future studies into this important topic.

6.2 Aims

The primary aim of this study is to define the changes in anal sphincter function following anterior resection and chemoradiotherapy using HRAM (compared with normal values and pre-treatment values).

The secondary aims are to correlate values for HRAM parameters following treatment with clinical variables, and with the presence of minor and major LARS.

6.3 Methods

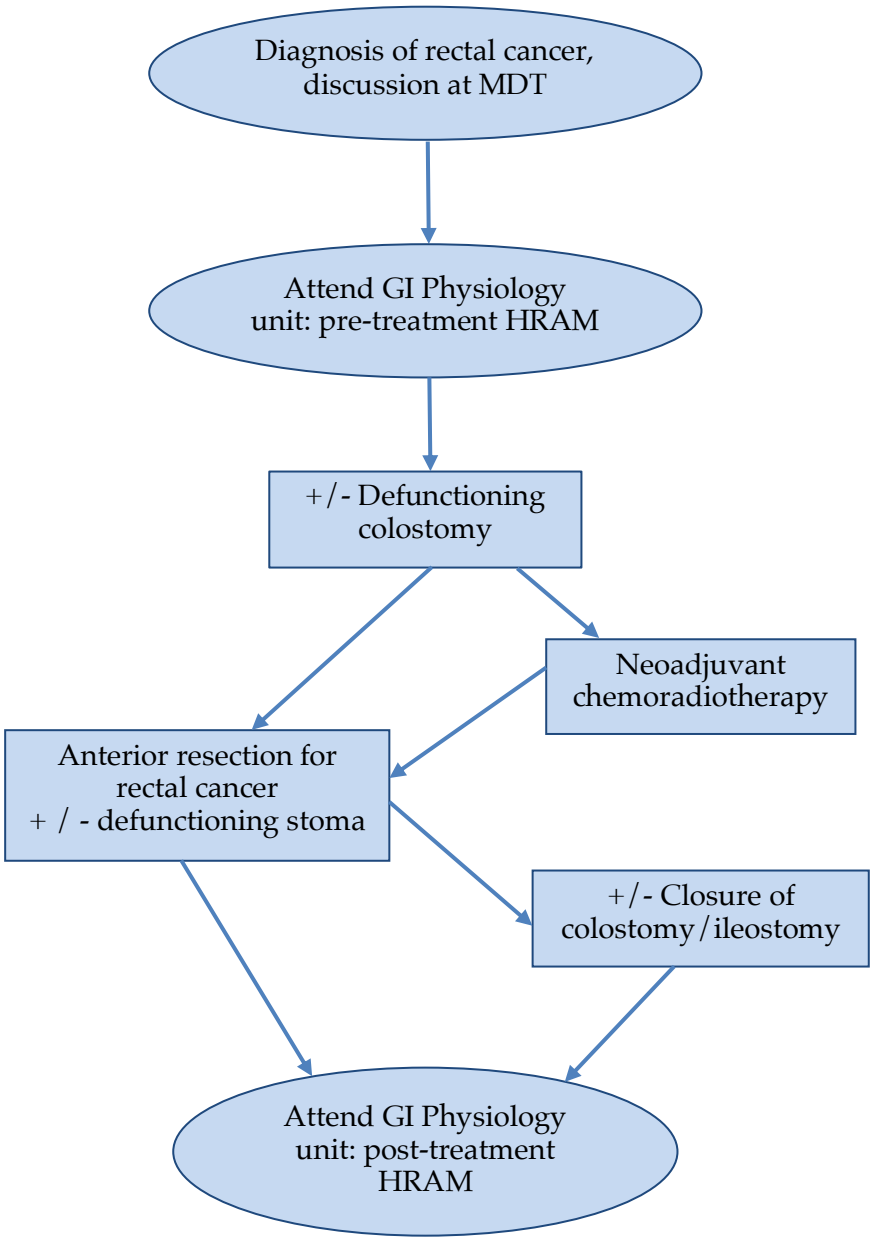
6.3.1 Patients

This was a single institution prospective cohort study. A change in clinical practice was introduced in August 2014 with all rectal cancer patients undergoing routine anorectal manometry as part of their pre-treatment planning. The aim of this was to

assess sphincter function prior to CRT or surgery. Patients were also sent for post-treatment testing as part of assessment of their functional outcomes. Figure 6.5 shows the flow of patients between pre treatment and post treatment testing.

As this was an exploratory study, the inclusion criteria were deliberately broad. Inclusion criteria included 1) Patients aged >18 years with biopsy-proven diagnosis of rectal cancer 2) Treatment plan from colorectal multidisciplinary team meeting (MDT) for patient to undergo chemoradiotherapy (CRT) or surgical resection 3) Able to provide informed consent to manometry. All patients were reviewed at the colorectal cancer MDT as per usual practice.

Figure 6.5 Flow of patients through pre treatment testing, treatment for rectal cancer, followed by post treatment testing. MDT: multidisciplinary team meeting; GI: gastrointestinal; HRAM: high-resolution anorectal manometry.



Exclusion criteria were limited in order to capture data from as many patients as possible at multiple time points. Exclusion criteria included permanent stoma creation, recurrent cancer and inability to tolerate manometry evaluation.

6.3.2 Treatment

Some patients who were discussed at MDT and had obstructive or difficult to manage symptoms underwent defunctioning sigmoid or transverse loop colostomy prior to the start of treatment.

Rectal cancer patients underwent a CRT regime with external beam radiotherapy given as 45 Gy delivered in 25 fractions over 5 weeks (daily dose 1.8 Gy) and concomitant chemotherapy given as the oral 5-FU derivative capecitabine.

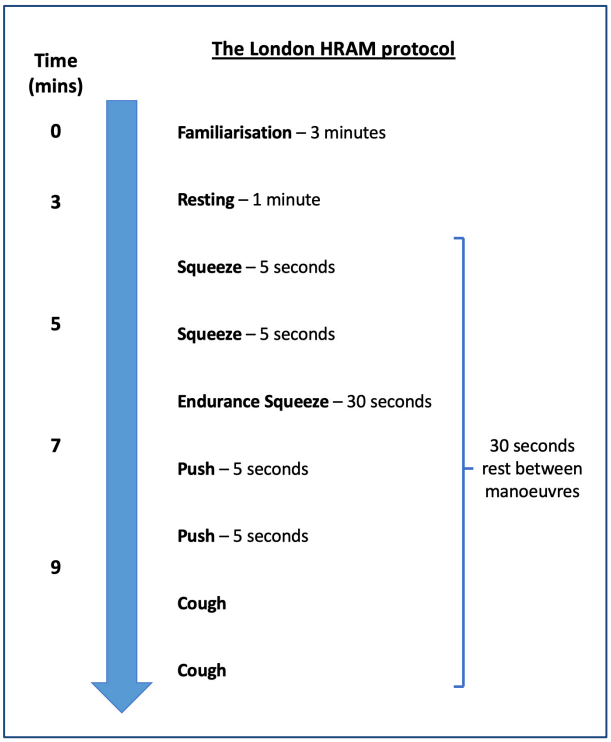
For patients who subsequently underwent resection of the rectal tumour, anterior resection was carried out according to the principles of total mesorectal excision, with histological analysis of the specimen. Defunctioning ileostomy was carried out selectively with the decision made intra-operatively by the surgeon depending mainly on the height of the tumour.

6.3.3 Manometry protocol

High-resolution anorectal manometry was carried out using standardised techniques[18] in accordance with a published protocol for HRAM at the Gastrointestinal (GI) Physiology Unit at The Royal London Hospital[22] shown in Figure 6.6. HRAM was performed using a solid-state catheter (UniTip: UniSensor AG, Attikon, Switzerland), of external diameter 12F. Data processing and analysis was carried out using a commercially available manometric system (Solar GI HRM v9.1; Medical Measurement Systems (MMS), Enschede, The Netherlands). HRAM assessment included measurement of functional anal canal length in cm, defined as: length of anal canal (cm) in which pressure exceeded rectal pressure by >5 mmHg[22].

Four measurements were used to report results; the definitions are taken from the paper defining normal values and measures used in HRAM by Carrington et al[22].
1. Average anal resting pressure defined as: average maximum pressure (mmHg) over the functional anal canal length (FACL) during the 1 min period of rest.
2. Maximum incremental anal squeeze pressure defined as: maximum recorded

Figure 6.6 HRAM protocol [22]



during voluntary squeeze, minus the mean maximum resting pressure prior to the manoeuvre (over 5 seconds).

3. Average incremental anal squeeze pressure defined as: mean maximum pressure (mmHg) sustained over the duration of the 5 second squeeze manoeuvre minus the mean maximum resting pressure prior to the manoeuvre (over 5 seconds).

4. Endurance squeeze index: FACL x average incremental anal squeeze pressure x 30 (seconds).

Physiology reporting was done as per standard department protocol. A written consent form was signed at the time of attending for manometry (as for all other patients undergoing this investigation). Manometry was carried out with the patient in the left lateral position; no bowel preparation was used prior to examination.

Following manometry, rectal volumes were assessed using a balloon attached to a Foley catheter introduced into the rectum. Three measurements were taken: first rectal sensation; then defaecatory desire volume and maximum tolerated volume (all in ml). Endoanal ultrasound was then used to assess the structural integrity of the anal sphincters. These additional tests are routinely used for patients undergoing HRAM testing within the GI Physiology Unit.

6.3.4 Questionnaires

The LARS questionnaire has been described in chapter 3. The LARS score includes five questions about bowel function and scores from 0–42[25]. Results were classified into three categories: no LARS (0-20); minor LARS (21-29) and major LARS (30-42). This questionnaire was used at the time of anorectal manometry, pre-treatment to record baseline data and post-treatment in order to assess functional outcomes.

Patients also underwent scoring with the St Marks Incontinence Score (SMIS), also known as the Vaizey score[26] (Appendix 5). This validated questionnaire includes seven questions in total, three related to the frequency of incontinence for solid stool, liquid stool and flatus, one relating to the frequency of bowel symptoms affecting lifestyle and three further yes/no questions about urgency and use of pads or constipating medication. Patients also completed the Cleveland Clinic Constipation Score (CCCS)[27] questionnaire (Appendix 6). This validated questionnaire includes eight questions about various aspects of constipation including frequency of bowel movements and the presence of abdominal pain.

The SMIS and CCCS questionnaires were included as they are routinely used prior to testing for all patients attending the GI Physiology Unit.

6.3.5 Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS Inc. Chicago, USA, version 22.0) and a two-sided p-value of <0.05 was considered statistically significant. Fisher's exact test was used to compare proportions for baseline categorical variables. This was an exploratory single centre study and therefore no sample size calculation was carried out. The sample size of 50 participants was determined as being a feasible sample size and a sample that would give meaningful results. Descriptive statistics including mean, median, percentages, range, interquartile range and odds ratios (OR) with 95% confidence intervals (CI) are reported. No adjustments were made for multiplicity of testing.

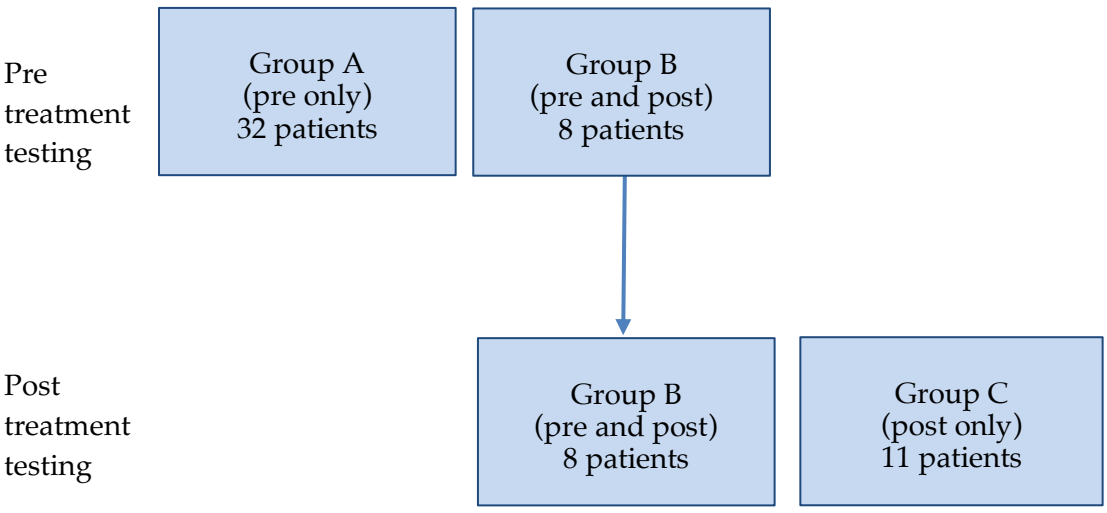
Scoring of the LARS questionnaire was carried out according to the original source of the score and patients were classified into three groups: no LARS, minor LARS, major LARS. Scoring of the SMIS and CCCS questionnaires was also carried out according to the sources of these scores. Values for post-treatment HRAM parameters were compared against pre-treatment values using the independent-samples T test and against values for the normal population using a one-sample T test. Results for patients from group B, who all underwent testing pre and post treatment (see below and Figure 6.7) were compared using a paired-samples T test. Chemoradiotherapy was treated as a dichotomous variable: no treatment vs. treatment. Age was treated as a continuous variable and dichotomised to above or below the median age of the respondents. Binary logistic regression was used to assess the impact of clinical and pathological variables on manometry parameters.

6.4 Results

6.4.1 Patients

52 patients met the inclusion criteria and were referred for HRAM between August 2014 and March 2018. 1 patient, with a low rectal cancer, which was a large tumour invading the sphincter complex, was not able to tolerate the tests. Therefore in total 51 patients were included in the analysis. Due to the fact that this protocol was newly introduced to the department, not all patients were referred for testing at each relevant time point. 32 underwent testing at diagnosis only, 8 underwent tests both pre-treatment and post-treatment and a further 11 patients only had tests following treatment. These three groups of patients were labelled groups A, B and C in order to clarify the results of analysis, see Figure 6.7. There were no complications arising from manometry testing.

Figure 6.7 Division of patients into three groups according to which set or sets of tests they underwent



6.4.2 Baseline data

51 patients were included in the analysis, 30 men (59%) and 21 women (41%). The median age was 66 years (range 28-88). Table 6.1 shows that the different groups were matched for baseline characteristics, except for the proportion with a stoma.

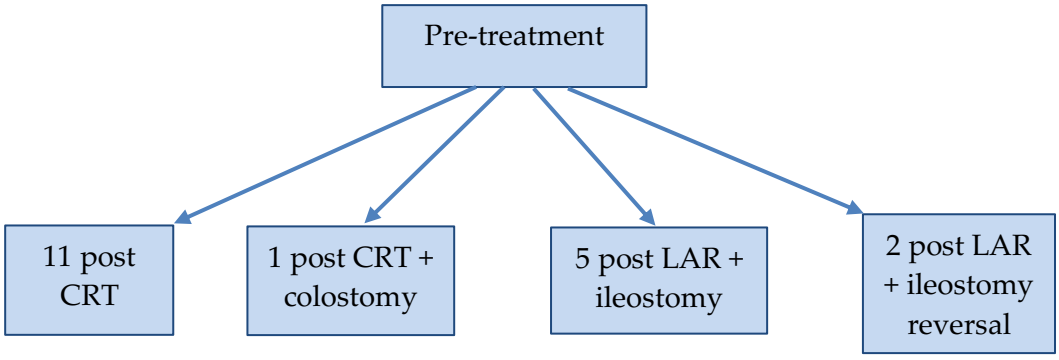
Table 6.1 Patient characteristics for groups A, B and C. IQR: interquartile range, CRT: chemoradiotherapy, LAR: low anterior resection.

	Group A (n = 32)	Group B (n = 8)	Group C (n = 11)	p-value
Female : Male	13 : 19	4 : 4	4 : 7	0.845
Median age (IQR) years	67 (58-71)	55 (37-63)	68 (51-72)	0.252
CRT : LAR	n/a all pre treatment	3 : 5	9 : 2	0.074
Stoma : no stoma at post treatment testing	n/a all pre treatment	5 : 3	1 : 10	0.041

6.4.3 Treatment

In total, 19 patients underwent HRAM testing following treatment. 12 patients had chemoradiotherapy as their primary treatment, only 1 of these had creation of a colostomy prior to therapy and still had their colostomy at post-treatment testing. 7 patients with rectal cancer had a low anterior resection (LAR) as their primary treatment for cancer, 5 of these still had their ileostomy at the time of post-treatment testing and 2 had undergone closure of ileostomy. These groups are summarised in Figure 6.8.

Figure 6.8 Diagram showing number of patients undergoing each treatment. CRT: chemoradiotherapy; LAR: low anterior resection.

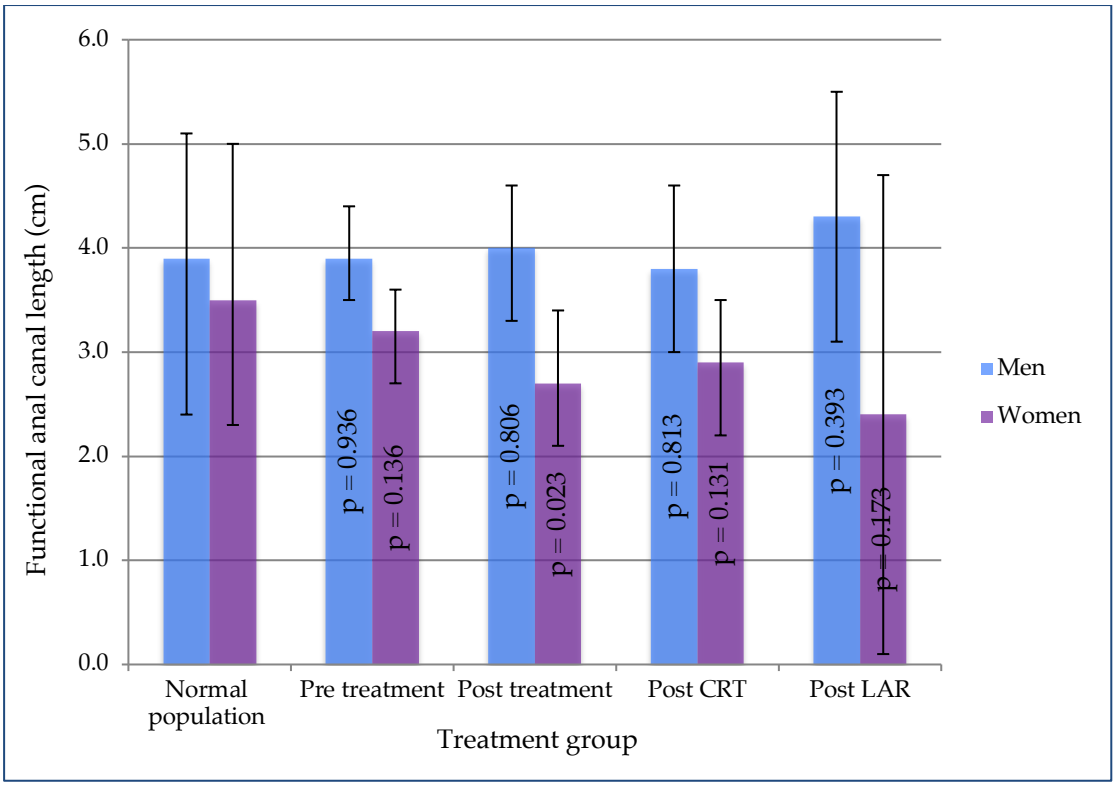


6.4.4 Results for functional anal canal length

The results for functional anal canal length (FACL) showed greater change in women than in men. For female patients, post treatment measurements were significantly lower than the normal population values, p = 0.023. For men, there was no significant

difference between any group and the normal population. These results are shown in Figure 6.9.

Figure 6.9 Functional anal canal length in the normal population (error bars for normal population demonstrate range) and in other groups vs. the normal population (error bars show 95% confidence intervals). CRT: chemoradiotherapy; LAR: low anterior resection.



There was no significant change in FACL between pre and post treatment measurements in either male or female patients. In men pre treatment mean was 3.9cm (95% CI 3.5 – 4.4cm) and post treatment mean 4.0cm (95% CI 3.3 – 4.6cm), p = 0.882. In women pre treatment mean was 3.2cm (95% CI 2.7 – 3.6cm) and post treatment 2.7cm (95% CI 2.1 – 3.4cm), p = 0.226. There was also no difference in FACL following CRT or LAR compared with pre treatment values, in either men or women.

6.4.5 Results for average anal resting pressure

There was no significant difference between the average anal resting pressure in all groups, either pre or post treatment when compared with normal population data, in

men or women. There was also no difference found on comparison between pre and post treatment testing results.

6.4.6 Results for maximum incremental anal squeeze pressure

The results for comparison with normal values for maximum incremental anal squeeze pressure show that for female patients, post treatment measurements were significantly lower than the normal population values in all patients post treatment, post CRT and post LAR groups. However, in contrast with FACL, the values for maximum incremental anal squeeze pressure prior to treatment in women were also significantly lower than normal values. For men, there were no significant differences between normal values and any group. These results are shown in Table 6.2.

Table 6.2 Results for maximum incremental anal squeeze pressure in different groups compared with population normal data[22]. 95% CI: confidence intervals; CRT: chemoradiotherapy; LAR: low anterior resection.

	Mean value (mmHg) male patients (95% CI)	p-value vs. normal	Mean value (mmHg) female patients (95% CI)	p-value vs. normal
Normal population	219 (range 61 – 525)	-	164 (range 45 – 324)	-
Pre treatment (groups A & B)	180 (134 – 226)	0.091	114 (80 – 148)	0.006
Post treatment (groups B & C)	251 (163 – 340)	0.433	98 (78 – 118)	<0.001
All post CRT	266 (154 – 378)	0.442	92 (68 – 116)	0.004
All post LAR	226 (63 – 388)	0.907	108 (69 – 147)	0.025

There was no significant difference in either men or women when comparing pre treatment and post treatment results.

6.4.7 Results for average incremental anal squeeze pressure

The results for comparison with normal values for average incremental anal squeeze pressure showed a similar pattern to those seen above. For female patients, pre-

treatment values were significantly lower than the normal population as were measurements in all patients post treatment and the post CRT group. For men, there were no significant differences between normal values and any group. These results are shown in Table 6.3.

Table 6.3 Results for average incremental anal squeeze pressure in different groups compared with population normal data[22]. 95% CI: confidence intervals; CRT: chemoradiotherapy; LAR: low anterior resection.

	Mean value (mmHg) male patients (95% CI)	p-value vs. normal	Mean value (mmHg) female patients (95% CI)	p-value vs. normal
Normal population	144 (range 40 – 366)	-	113 (range 29 – 235)	-
Pre treatment (groups A & B)	122 (89 – 154)	0.170	70 (48 – 91)	0.001
Post treatment (groups B & C)	187 (120 – 254)	0.180	74 (52 – 95)	0.003
All post CRT	201 (118 – 284)	0.227	66 (45 – 87)	0.013
All post LAR	164 (33 – 294)	0.668	86 (23 – 149)	0.209

On comparison between pre treatment and post treatment values, there was no significant difference in men and women for the majority of groups. In male patients there was a trend towards measurements being higher in the post treatment groups than on the pre treatment measurement. This was the case for all patients, post CRT and post LAR patients but was only significant in the rectal cancer patients with a pre treatment mean value of 122mmHg (95% CI 89 – 154) compared with 187mmHg (95% CI 120 – 254) following treatment, p = 0.040.

6.4.8 Results for endurance squeeze index

The results for endurance squeeze index were similar to those for maximum and average incremental anal squeeze pressures. For female patients, pre treatment measurements were significantly lower than the normal populations (p = <0.001), as were post treatment measurements for all patients (p = <0.001) and post CRT groups (p = 0.001). For men, pre treatment (p = <0.001) values were significantly lower than

those for the normal population but there was no difference between post treatment values and the normal population.

As for average incremental anal squeeze pressure, in male patients there was a trend towards measurements being higher in the post treatment groups than on the pre treatment measurement. This was significantly different for the overall patient group with a pre treatment mean value of 2239mmHg/s (95% CI 1396 – 3081) compared with 3781mmHg/s (95% CI 2766 – 4796) following treatment, p = 0.028. This pattern was not repeated in female patients.

6.4.9 Results for sensitivity testing

Testing for rectal sensation included three measurements, first rectal sensation, defaecatory desire volume and maximum tolerated volume. The results for all three measurements showed similar patterns. In both male and female patients post treatment measurements were significantly lower than the normal population values in all patients, post CRT and post LAR groups. This is consistent with rectal hypersensitivity. In men the pre treatment values were normal but in women, the pre treatment test results also showed values significantly lower than normal. These results are shown in Figures 6.10 and 6.11.

There was no significant difference between pre treatment values and post treatment values for rectal sensation in male or female patients across the three different measurements used.

Figure 6.10 Rectal sensation (ml) for men in the normal population and in other groups vs. the normal population. CRT: chemoradiotherapy; LAR: low anterior resection.

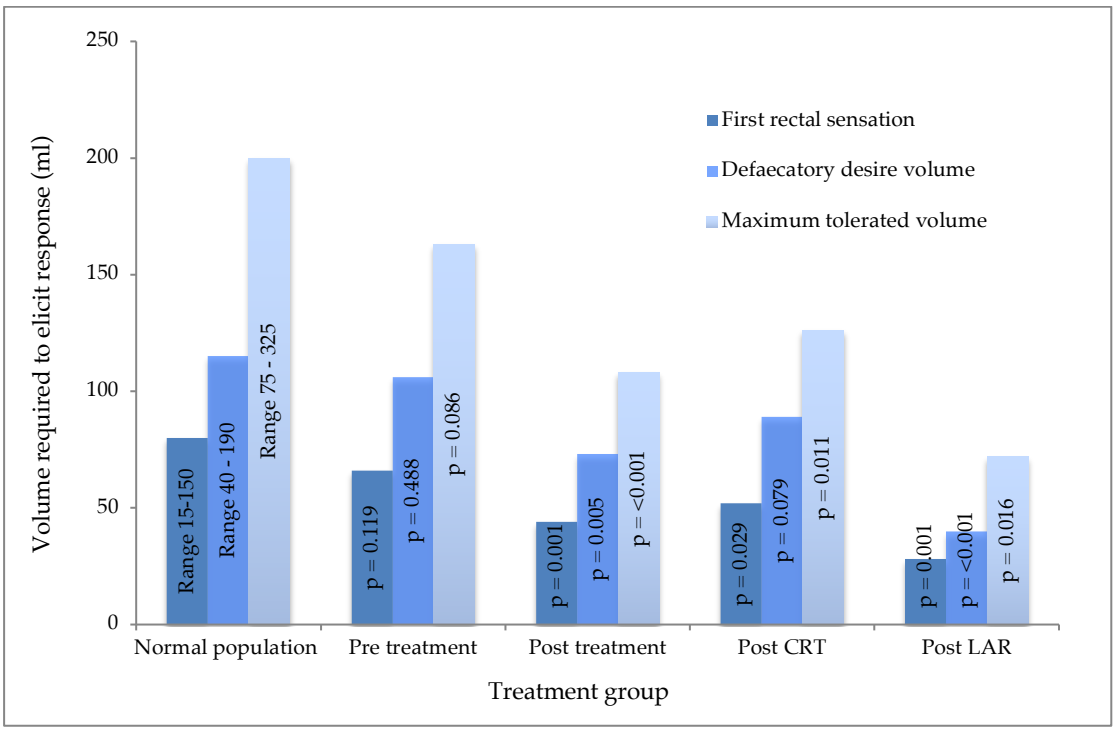
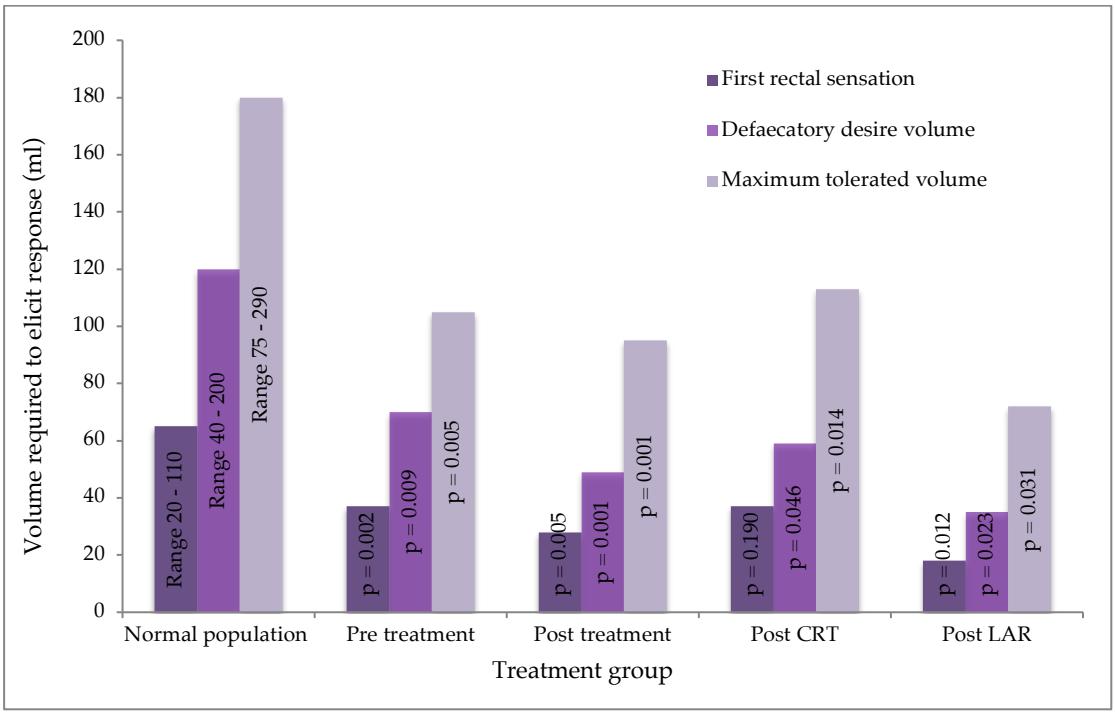


Figure 6.11 Rectal sensation (ml) for women in the normal population and in other groups vs. the normal population. CRT: chemoradiotherapy; LAR: low anterior resection.



6.4.10 Results for endoanal ultrasound

33 patients underwent pre treatment ultrasound, 20 men and 13 women. 18 patients had an ultrasound following their treatment, 11 of these were male. The majority of rectal cancer patients had normal sphincter morphology on ultrasound. These figures are shown in Table 6.4.

Table 6.4 Pre and post treatment endoanal ultrasound findings. USS: ultrasound.

	Normal n (%)	Abnormal n (%)
Pre treatment USS:		
External sphincter	25 (75.8%)	8 (24.2%)
Internal sphincter	20 (60.6%)	13 (39.4%)
Post treatment USS:		
External sphincter	14 (77.8%)	4 (22.2%)
Internal sphincter	12 (66.7%)	6 (33.3%)

Of those with an abnormal pre treatment USS, the majority had defects or distortion directly due to tumour. On post treatment USS, 50% of abnormalities were described as being directly due to tumour with the remainder being characterised as simple defects or due to atrophy, scarring or fibrosis.

Between male and female patients there was no difference in the numbers with abnormal pre or post treatment USS. Female gender was a risk factor for abnormal external sphincter on USS pre treatment with an odds ratio of 7.714 (95% CI 1.246 to 47.754, p = 0.028) and was also a risk factor for abnormal external sphincter on USS post treatment with an odds ratio of 6.000 (95% CI 1.172 – 30.725, p = 0.032). There was no difference identified in the number of patients with abnormal post treatment ultrasound in patient groups that underwent CRT or LAR.

6.4.11 Questionnaire results

27/51 patients completed the SMIS questionnaire prior to their treatment. The most frequent score was 0 (37.0%), with a median score of 3 (IQR 0 - 5). Only 2 patients scored ≥12 indicating moderate incontinence. Following treatment, 9/51 (only 17.6% of the total cohort) completed the SMIS questionnaire. The median score was 2 (IQR

0 - 13). The maximum score was 17 and 3 (33.3%) of the patients scored ≥12. On statistical testing there was no difference between pre and post treatment scores (p = 0.236).

On pre treatment testing, patients with SMIS ≥12 did not have significantly different manometry measurements from those with SMIS scores <12. In patients with SMIS ≥12 post treatment, the average anal resting pressure was significantly reduced compared with those with SMIS <12 (44 mmHg vs. 72 mmHg, p = 0.018, there was no difference seen in other manometry variables. There was no statistically significant difference in FACL or sensitivity measurements between those with high or low SMIS scores.

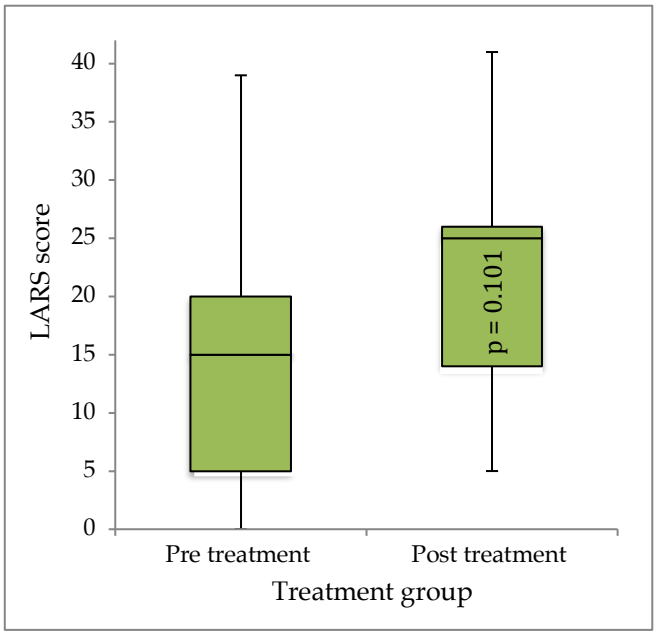
27/51 patients completed the CCCS questionnaire prior to treatment. For these patients, the median score was 2 (IQR 2 - 4). No patients in this cohort scored ≥15, a score that indicates moderate constipation. Following treatment 9 patients completed this questionnaire. The median score was 4 (IQR 2 - 7). The maximum score was 10 and again no patients scored ≥15.

There was no difference between pre and post treatment CCCS scores p = 0.432. Scores were dichotomised to above and below the median score of 3 (as no patients scored ≥15 this could not be used to dichotomise). In patients with above and below median scores there was no difference in pre or post treatment FACL, manometry measurements or sensitivity.

22/51 patients (43.1%) completed the LARS score pre treatment. The median score was 15 (IQR 5-20). The majority of patients, 18 (81.8%) had no LARS; 2 (9.1%) had minor LARS and 2 (9.1%) had major LARS prior to treatment. Only 6 (11.8%) completed the questionnaire following their treatment. The median score was increased to 25 (IQR 14 - 26). 2 (33.3%) patients had no LARS, a further 3 (50%) had minor LARS and 1 (16.7%) had major LARS.

Although the LARS scores were increased post treatment when compared with pre treatment values, this difference was not significant (p = 0.101). This is illustrated in Figure 6.12. With scores dichotomised to no LARS or minor/major LARS, there was no difference between groups in pre or post treatment FACL, manometry measurements, sensitivity or proportion with abnormal USS.

Figure 6.12 Difference between LARS score pre and post treatment. The middle line in each box represents the median value, with the boundaries of the box representing the quartiles and whiskers showing minimum and maximum values. LARS: low anterior resection syndrome.



For all three questionnaires used, scores prior to treatment were not dependent on gender and scores following treatment were not dependent on gender, use of CRT or whether patients had undergone LAR.

6.5 Discussion

This study showed that in a department where HRAM is accessible it is easily incorporated into pre and post treatment assessment for patients with rectal cancer. These tests were widely accepted; no patients refused to have testing carried out and only two patients did not tolerate testing. No complications arose as a result of these tests. There are several benefits of HRAM. It is intuitive and easy to learn, its reproducible and objective nature make it an ideal tool for research and to measure changes pre and post intervention. The visual output is easy for patients to understand and follow, meaning it can be used for biofeedback and it is also easily understood by healthcare professionals who do not have specific knowledge of HRAM.

This exploratory study has established the feasibility and application of HRAM as a tool to assess functional changes following treatment for rectal cancer. This is a novel area of research as, despite the advantages of HRAM outlined above, there are very few studies that have used HRAM to assess physiological changes in rectal cancer patients. There is also a lack of studies demonstrating the clinical utility and potential

benefits of HRAM when compared with standard manometry[28]. Even conventional anorectal manometry has a lack of evidence for clinical benefit and conflicting evidence about how well it correlates with symptoms[5,24]. One study has successfully used HRAM to assess the effect of radiotherapy and anterior resection in rectal cancer patients and found that functional outcomes were worse in patients who had undergone both CRT and LAR with HRAM showing low resting pressures, reduced capacity and compliance in these patients[29].

Functional anal canal length correlates with both resting and squeeze pressures and is a measurement that can therefore reflect anal sphincter function[30]. It has also been shown to correlate with incontinence scores[31] and improvement following sphincter repair predicts improvement in continence[32]. Average FACL is known to be shorter in women than in men[22]. The current study has demonstrated that in women with rectal cancer, post treatment FACL is below normal. FACL was also reduced following CRT and LAR, although not significantly, and this shortening may be a potential mechanism by which these treatments impact on sphincter function.

This study did not show any difference in anal resting pressure following treatment for rectal cancer. There was also no difference found when comparing the groups in the study with normal population data. There was a possible reduction in resting pressures in men post LAR but insufficient numbers to really draw any conclusions regarding this. Previous studies have shown conflicting results in this area. Decreased resting pressures have been shown in patients with LARS and those with incontinence[2,5]. Other studies have shown no difference in resting pressures following anterior resection[6]. Reduced anal resting pressures were identified in patients scoring highly on the SMIS questionnaire and therefore experiencing incontinence. This would be expected, as resting pressure is essentially a measure of internal anal sphincter function, a crucial mechanism for controlling continence[33].

On HRAM measures of squeeze there were again some differences identified between genders which have not previously been reported. The average and maximum incremental anal squeeze in women showed that pre and post values were both lower than normal but no difference was seen between pre and post measurements. Men essentially had normal results for these two measures. Both genders showed lower than normal results for endurance squeeze index prior to treatment, with women also showing low values post treatment. This is in keeping with previous studies, which have shown decreased squeeze pressures following

anterior resection[2] and coloanal anastomosis[34]. Some interesting patterns were seen with some of the measures for squeeze having higher post treatment values in men when compared with pre treatment values. This may be due to the effect of the tumour during pre treatment testing but other unknown mechanisms may also be involved. Larger studies would be required to clarify these patterns.

Marked abnormalities of rectal sensation were seen following treatment with both CRT and LAR, with low sensory thresholds, indicating rectal hypersensitivity. This could clearly account for urgency, which is one of the most troublesome symptoms following treatment for rectal or anal cancer. There is good evidence from previous studies showing that altered rectal sensation plays a part in dysfunction following anterior resection[5,10,35,36,49]. This altered sensitivity persists over time and may account for the lack of improvement in symptoms beyond one year post anterior resection[10].

Rectal hypersensitivity is likely to result from a combination of reduced rectal reservoir, and also altered compliance of the neorectum[5]. Rectal compliance is defined as "change in volume or cross-sectional area divided by the change in pressure"[37]. It cannot be measured using manometry alone; it has traditionally been assessed using a barostat[38] but can also be measured using balloon distension of the rectum with concurrent pressure measurement[29]. Creation of a colonic pouch with side to end anastomosis at anterior resection leads to a temporary improvement in post-operative symptoms when compared with end to end anastomosis[39] and this is believed to be partly due to better capacity and compliance[39].

Although altered reservoir and compliance may partly explain changes in sensitivity following anterior resection, these mechanisms cannot account for abnormalities following CRT alone. Similar findings of rectal hypersensitivity have been previously shown following CRT[40]. The pathophysiology behind the detrimental effect of radiotherapy on anorectal function is not fully understood[41]. Radiotherapy is known to induce fibrosis and scarring within the sphincters[42]. Reduced compliance is believed to be one of the main mechanisms by which radiotherapy alters anorectal function, causing rectal wall stiffness and poor distension with reduced capacity[43]. For patients who undergo CRT followed by anterior resection, the neoadjuvant radiotherapy can make their surgery more challenging due to fibrosis[41]. Patients undergoing both radiotherapy and surgery for rectal cancer have been shown to have significantly lower anal resting and squeeze pressures[29,42] and one study of 13 patients undergoing CRT alone, in the context of anal cancer, showed similar

findings[44]. However, in the current study no significant change in resting or squeeze pressures was seen following CRT.

The results of this study showed that the majority of abnormalities seen on endoanal ultrasound were directly due to tumour, although these findings have to be interpreted in light of the fact that the assessor was aware of the patient's clinical presentation. Female gender was identified as a risk factor for abnormal ultrasound post treatment. Although gender differences in endoanal ultrasound findings have been shown previously[45], this specific finding has not previously been reported.

The results from all three pre treatment questionnaires indicate that patients had good overall function prior to treatment, even though, for women particularly, many of the pre treatment manometry results were significantly different from the normal population. The small number of patients that completed the LARS questionnaire post treatment limited the ability to meet the stated aim of correlating LARS with manometry findings. With a larger cohort, the hypothesis that those with LARS have hypersensitivity and lower resting and squeeze pressures on HRAM than patients with no LARS could be tested. The results did suggest that there was an increase in the LARS score following treatment, but this did not reach significance. It would not be an unexpected finding, especially since most patients underwent testing only a short time following their treatment. Two studies have previously explored the relationship between LARS score and manometry results. The first of these included 65 patients and demonstrated decreased resting anal sphincter pressures, decreased rectal volume tolerability and decreased rectal compliance in patients with major LARS compared with those with no/minor LARS[5]. A further study of 28 patients also found that resting anal pressure in those with major LARS was significantly lower than no LARS group, maximum squeeze pressure was also reduced[46].

Although outside of the remit of this study, one of the potential uses for HRAM will be in the assessment of patients who develop LARS following anterior resection. Investigation with HRAM including sensitivity testing and endoanal ultrasound, in individual patients, is an ideal tool for highlighting specific pathology, which may be amenable to therapy. Pelvic floor rehabilitation including biofeedback has been shown to be an effective treatment for LARS[47]. One Chinese study has used HRAM as a tool to assess the effectiveness of biofeedback as therapy for LARS[48] but so far, no studies have used HRAM as a tool to guide choice of therapy for LARS. A further systematic review of the literature on the treatment of LARS concluded that current

treatment is based on weak evidence with low quality studies, and that well conducted multicentre randomised trials are urgently needed in this area[18].

The demographics of the cohort reflect those of the population of rectal cancer patients[50]. This study does, however, have several major limitations. Due to the exploratory nature of the study, it was conducted at a single centre. As the testing protocol was newly introduced into the department, many patients did not undergo testing at all relevant timepoints. This was partly because not all of the clinical staff were familiar with the protocol and patients were not referred at all time points when they should have been. It is also likely that some eligible patients were not referred at all. Particularly around the time of diagnosis, the management of the cancer itself may understandably have taken priority for clinicians and not allowed time for patients to attend for manometry prior to starting treatment, this problem would be likely to affect any study of this type. Not all patients attended their appointment as some were concerned about the nature of the tests and had not been given sufficient information. Due to the protocol being newly introduced, some patients had already undergone their treatment at this point and only underwent post treatment tests. The end date of the study was dictated by the constraints of the timeframe of the thesis and some patients had only undergone pre treatment testing at this point.

The LARS, SMIS and CCCS questionnaires were unfortunately only completed by limited numbers of patients rather than the whole cohort. This was due to some confusion amongst physiology staff who were carrying out the testing about whether the new protocol included the questionnaires or not, as well as lack of familiarity with the LARS score which was newly introduced to the department.

As a result of these difficulties, the cohort was heterogeneous, and made up of patients at different time points, undergoing different therapies. This led to small numbers in some of the patient groups, which raises the possibility of type II errors and also limited the analysis. It was not possible to carry out some analysis that would have been interesting, for example the effect of a defunctioning stoma on manometry results, about which little is known. No patients underwent CRT followed by LAR and then testing, so it was not possible to examine the cumulative effect of CRT and LAR. Assessors carrying out physiology testing were aware of the patient's clinical history and whether they had undergone treatment or not, which may introduce unconscious bias into reporting of results. Testing post treatment was

carried out at varying lengths of time following treatment and this was not standardised in any way. As discussed in chapter 3, it is known that function following anterior resection is most troublesome during the initial 12-month period[51] and the results of post treatment testing may well have been different if carried out after the first year.

This study has noted some interesting patterns with abnormalities identified both prior to and following treatment. Some of these are consistent with findings from the literature as outlined above. In order to examine in more detail, the effect of anterior resection specifically, patients would need to be tested prior to treatment, following neoadjuvant chemoradiotherapy and following anterior resection, after a suitable time period. These patients could also have their manometry and other test results correlated with their LARS score and specific symptoms including urgency. Patients with LARS could undergo biofeedback or retraining using HRAM as an assessment method for this. The aim of such a study would be to use HRAM specifically to better understand LARS and find potential targets for biofeedback therapy. HRAM is not widely or routinely used yet in the UK, and is only available at tertiary centres. At the current time, therefore, it would be difficult to introduce it as a routine part of care for rectal cancer patients and this limits the applicability of research in this area. However, patients who develop significant problems with LARS following anterior resection can be referred into tertiary centres to undergo assessment and treatment and HRAM results are easily presented in a format that is understandable to the team at the referring centre.

Clinicians caring for patients with rectal cancer need to understand the potential impact that therapies can have on anorectal function and continue work to understand the pathophysiological mechanisms involved. Measures to limit and prevent dysfunction should be taken wherever possible, including tailoring of radiotherapy fields, careful surgery to limit nerve damage and minimising direct injury to sphincters. It remains to be seen how developments in rectal surgery, including transanal approaches, will impact functional outcomes in the future. Direct visualisation of the pelvic nerves may be improved but prolonged instrumentation may have unforeseen effects.

6.6 Conclusions

HRAM is an ideal tool for the routine pre and post treatment assessment of sphincter function in patients with rectal cancer. This exploratory study has established the feasibility of this investigative technique for studying functional changes following treatment for rectal cancer.

Patients undergoing treatment for rectal cancer have likely abnormalities on testing of functional anal canal length, HRAM, rectal sensitivity and endoanal ultrasound but correlation of these findings with symptoms is crucial and requires further study. Large-scale multicentre prospective studies, carefully designed to answer specific questions, are needed and these should incorporate HRAM both as a means of assessment and as a research tool to assess the effectiveness of interventions for LARS.

HRAM has the potential to help us better understand functional changes following treatment for rectal cancer and how these functional changes are linked to the symptoms that patients experience, an understanding that could ultimately help to achieve the aim of preserving sphincter function during treatment for rectal cancer.

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Chapter 7. Conclusions

The assessment and management of rectal cancer is complex, requiring consideration of multiple interacting factors and coordinated care from several specialities. The range of available therapeutic options is broad, and expanding. Surgical techniques continue to evolve and these developments will require ongoing research to guide decision-making. Our selection of neoadjuvant therapy is dependent on the accuracy of the investigative tools we have available and currently remains an imperfect science.

Oncological outcomes for rectal cancer are improving and, as a result, over the last few years there has been a clear shift in the paradigm of care. The focus is no longer necessarily on sphincter preservation, but on the preservation of acceptable anorectal function. This is an important factor in determining the long-term quality of life of survivors. This thesis therefore aimed to discover what determines the ability to preserve functioning sphincters during the management of low rectal cancer.

The questionnaire study in chapter 3 has confirmed that our current management leads to morbidity for many patients. Low Anterior Resection Syndrome (LARS), with disordered bowel function, affects a considerable proportion of patients in the UK following anterior resection, it is detrimental to quality of life and does not resolve with time. The risk factors identified for LARS in this study are neoadjuvant radiotherapy, female gender, younger age and defunctioning stoma. The assessment of functional outcomes must become a routine part of follow-up to ensure that we avoid doing a disservice to those who could benefit from treatment for LARS.

There has been improvement in rectal cancer care but the next step forward will be tailored delivery of care for the individual, based on patient factors and tumour biology. Along with ensuring the best possible oncological outcome, personalisation of care should be a specific aim of the multi-disciplinary team. Response to neoadjuvant chemoradiotherapy is variable, and it is not currently possible to predict tumour response on an individual level. As shown in chapter 3, neoadjuvant radiotherapy is a risk factor for poor functional outcome. Tools to predict response to neoadjuvant therapy would rationalise its use, thereby improving functional outcomes for those patients who would avoid radiotherapy that would not be of benefit to them. This targeting of therapy will need reliable biomarkers, for use either individually or as part of a panel allowing stratification of patients.

Difficulties interpreting the effects of chemoradiotherapy on a tumour and its surrounding tissues have led to innovation in imaging techniques. Some of these, including diffusion weighted MRI (DWI), are functional imaging techniques, with the ability to go beyond assessing anatomy, to defining the metabolic characteristics of a tumour. It is likely that the use of such technology will continue to grow and become increasingly important in the initial planning of therapy and prediction of response to neoadjuvant therapy. The results of the imaging study in chapter 4 show that use of DWI is feasible and that measures derived from DWI have potential as biomarkers, not just to assess, but also to predict, response to neoadjuvant therapy. Following improved standardisation of techniques and reporting, combined with validation, these measurements could be incorporated in multi-disciplinary decision-making.

The use of molecular biomarkers will be one of the most important developments across cancer care in the coming years. The laboratory study in chapter 5 has demonstrated that analysis of microRNA expression from pre-treatment rectal biopsies is feasible and that the expression of microRNAs potentially differs between responders and non-responders to neoadjuvant therapy. MicroRNA targets therefore have potential as predictive biomarkers for response to neoadjuvant therapy in rectal cancer. The study identified three possible microRNA biomarkers. Following further validation studies, these could in the future be combined with other molecular or imaging biomarkers. Breast cancer patients already benefit from a panel of biomarkers used to assess the likely response of their tumour to chemotherapy and a similar stratification for neoadjuvant therapy in rectal cancer would benefit patients.

With the focus on preservation of anorectal function, the option of organ preservation for selected patients with rectal cancer, with avoidance of surgery altogether, is being increasingly explored and studied. Questions remain about the long-term oncological outcomes and the most appropriate surveillance schedule, and anterior resection remains the current gold standard. We need to understand more about the impact of chemoradiotherapy, when used in isolation, on sphincter physiology and functional outcomes in order to better counsel and advise patients about the options available.

The findings in Chapter 6 outline the potential of high-resolution anorectal manometry (HRAM) to further our understanding of the factors underlying dysfunction following chemoradiotherapy and anterior resection. This exploratory

study has established the feasibility of this investigative technique for studying functional changes following treatment for rectal cancer. Studies utilising HRAM would also have the potential to help us understand how functional changes are linked to the symptoms that patients' experience, an understanding that could ultimately help to achieve the aim of preserving sphincter function during treatment for rectal cancer. HRAM is an ideal tool for the routine pre and post treatment assessment of sphincter function in patients with rectal cancer and is also ideal for use as a research tool to assess the effectiveness of interventions for LARS.

Variations in the management of rectal cancer across the UK reflect gaps in our knowledge about the 'best' care for each patient; further research is undoubtedly needed in many areas. Better understanding of decision making by individual clinicians, and at team level, is one challenging but important aspect of this research. An ideal way to study the many facets of rectal cancer care would be a large prospective multicentre cohort study of patients with rectal cancer. Following their course from diagnosis through investigation and treatment, with appropriate functional, imaging, laboratory and manometry results would allow improved interpretation of complex interacting factors, decision-making, outcomes following treatment for rectal cancer as well as a more sophisticated understanding of the factors which affect these outcomes.

It remains the responsibility of individual clinicians to continue to challenge the lack of understanding, to ask questions and to demand better evidence in order to ensure that we continue to improve outcomes for all rectal cancer patients.

Appendix 1. Invitation Letter

INVITATION LETTER

Anterior resection syndrome following sphincter-preserving surgery

Chief Investigator: Mr Mohamed Thaha

Ethics Committee Reference: 14/EM/0117

Dear Sir or Madam

We would like to invite you to participate in a research study. This letter has been sent to you by your surgical team who are working with us on this study, they have access to your name and address but they have not sent any of these details to us. If you decide that you do not want to participate in the study simply do not return the enclosed questionnaire.

This study is looking at symptoms and quality of life following surgery to remove part of the bowel. Taking part in the study will involve completing one questionnaire and returning it to us in a pre-paid envelope.

We have enclosed a Participant Information Sheet which explains why we are doing this study, why you have been invited and other details about the study.

If you have any questions then please get in touch with us via the contact details above. Thank you for considering taking part in our study.

Yours sincerely

Kathryn Lynes
Colorectal Research Fellow
National Centre for Bowel Research and Surgical Innovation

Appendix 2. Participant Information Sheet

Blizard Institute
Centre for Digestive Diseases
Barts and The London School of Medicine and Dentistry

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INFORMATION SHEET

Project: Anterior resection syndrome following sphincter-preserving surgery

We would like to invite you to take part in a research study. Before you make your decision you need to understand why the research is being done and what it would involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

What is the purpose of the study?

After an operation to all, or part of, the rectum (the lower part of the large bowel), patients can sometimes have difficulties controlling their bowels. This can include symptoms such as urgency (uncontrollable urge to open your bowels) and faecal incontinence (inability to hold in stool resulting in soiling). Currently there is a lack of understanding of the exact cause of these symptoms.

Gaining information from patients who have already had these operations will allow us to further understand how many patients develop these problems, the impact they have on quality of life and how they vary according to different risk factors. This improved understanding will allow us to better predict when patients will experience these problems and help us tailor their treatment accordingly.

Why have I been invited?

You have been invited as your surgical team have identified that you have previously had surgery to remove all, or part of, your rectum.

Do I have to take part?

It is up to you whether you want to take part or not. If you do not want to be involved that is not a problem and will not affect any of your care in the future.

What will participation in the research involve in practical terms?

If you agree to participate in the study you will need to complete a questionnaire, which will take around 30 minutes. A pre-paid envelope is included for you to return the questionnaire to

us. If you complete and return the questionnaire, we will assume this means you have given us your consent to take part in the study.

What are the possible benefits of taking part?

You will not gain any personal benefit from being involved in the study. However, the information we get from this study will hopefully help improve the understanding of patients with these problems by determining how many patients are affected. The results will also help us to understand why some patients develop these symptoms and the impact that they can have on quality of life. This will help develop and test new treatments for these problems.

How will your confidentiality be protected?

This letter has been sent to you by your surgical team who are working with us to carry out this study. They have not provided us with your name, address or any other personal or medical information about you. The information from the questionnaire will be stored securely and confidentially. Your name will not be used or identified in the analysis of the information generated by this study.

How can I find out the results of the study?

If you would like to find out the results of the study then please contact us via the details below and we will be happy to provide a summary once the study is completed.

What happens if you are worried?

If answering the questionnaire makes you concerned about your symptoms then you should contact your medical team to discuss them. This may be your GP, your Colorectal Surgeon or your Colorectal Specialist Nurse.

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (see contact details above).

What happens if there is a problem?

We understand that some of the questions are personal in nature and ask about private information on your bowel habit and daily activities. The questions are important in order to find out about symptoms that patients experience. If you do not feel comfortable to answer the questions, either leave them blank or do not complete the questionnaire.

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you. Please contact the Patient Advisory Liaison Service (PALS) if you have any concerns regarding the care you have received, or as an initial point of contact if you have a complaint. You can find your nearest PALS office on the NHS Choices website at www.nhs.uk.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity.

Derby Research Ethics Committee has reviewed this study.

Appendix 3. Study Questionnaire

Blizard Institute
Centre for Digestive Diseases
Barts and The London School of Medicine and Dentistry

National Centre for Bowel Research and Surgical Innovation
1st Floor, Abernethy Building
2 Newark Street
London E1 2AT

QUESTIONNAIRE

Anterior resection syndrome following sphincter-preserving surgery

This questionnaire has three parts.

The first part asks details about your treatment, as background information to confirm for us that this study applies to you and help us interpret the results.

The second part asks about bowel function.

The third part asks general questions about your health and daily activities.

We would be grateful if you could complete all parts of the questionnaire but if there are any questions you do not feel happy to answer, then please leave them blank. There are no 'right' or 'wrong' answers. The information that you provide will remain strictly confidential.

Part 1 Background Information

What is your gender: Male ☐ Female ☐

Please provide your age: _____

Have you had surgery to remove all or part of your lower large bowel (rectum)?

Yes ☐ No ☐

If so, when was this surgery carried out _____

If you answered no, and you have not had this surgery then please do not complete the questionnaire as it does not apply to you, we apologise that you have been sent this questionnaire in error.

At the time of your surgery did you have a stoma created? Yes ☐ No ☐

If you answered yes, has this now been closed/reversed? Yes ☐ No ☐

If your stoma has been closed/reversed, when was this done _____

Following your original surgery have you had any other further operation (apart from stoma closure/reversal) Yes ☐ No ☐

If you answered yes, please provide details _____

_____

Other than monitoring your progress, are you still having any treatment for your cancer?

Yes ☐ No ☐

If you answered yes, please provide details _____

_____

Prior to surgery did you have treatment with:

Chemotherapy: Yes ☐ No ☐

Radiotherapy: Yes ☐ No ☐

If you had radiotherapy, how many days was this for? _____

After your surgery did you have treatment with:

Chemotherapy: Yes ☐ No ☐

Radiotherapy: Yes ☐ No ☐

Have you ever had radiotherapy for any other reason? Yes ☐ No ☐

If you answered yes, please provide details _____

_____

How was your surgery carried out?

Laparoscopically (keyhole surgery) ☐

Open surgery ☐

Planned laparoscopically, but converted to open during the operation ☐

Part 2 Bowel Function Questionnaire

Please tick only one box for each question. It may be difficult to select only one answer, as we know that for some patients symptoms vary from day to day. We would kindly ask you to choose one answer which best describes your daily life. If you have recently had an infection affecting your bowel function, please do not take this into account and focus on answering questions to reflect your usual daily bowel function.

1: Do you ever have occasions when you cannot control your flatus (wind)?

☐ No, never

☐ Yes, less than once per week

☐ Yes, at least once per week

2: Do you ever have any accidental leakage of liquid stool?

☐ No, never

☐ Yes, less than once per week

☐ Yes, at least once per week

3: How often do you open your bowels?

☐ More than 7 times per day (24 hours)

☐ 4-7 times per day (24 hours)

☐ 1-3 times per day (24 hours)

☐ Less than once per day (24 hours)

4: Do you ever have to open your bowels again within one hour of the last bowel opening?

☐ No, never

☐ Yes, less than once per week

☐ Yes, at least once per week

5: Do you ever have such a strong urge to open your bowels that you have to rush to the toilet?

☐ No, never

☐ Yes, less than once per week

☐ Yes, at least once per week

Part 3 Questions about your health

Please answer all of the questions by circling the number that best applies to you.

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or suitcase?	1	2	3	4
2.	Do you have any trouble taking a long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:		**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
6.	Were you limited in doing either your work or other daily activities?	1	2	3	4
7.	Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8.	Were you short of breath?	1	2	3	4
9.	Have you had pain?	1	2	3	4
10.	Did you need to rest?	1	2	3	4
11.	Have you had trouble sleeping?	1	2	3	4
12.	Have you felt weak?	1	2	3	4
13.	Have you lacked appetite?	1	2	3	4
14.	Have you felt nauseated?	1	2	3	4
15.	Have you vomited?	1	2	3	4
16.	Have you been constipated?	1	2	3	4

During the past week:		Not at All	A Little	Quite a Bit	Very Much
17.	Have you had diarrhea?	1	2	3	4
18.	Were you tired?	1	2	3	4
19.	Did pain interfere with your daily activities?	1	2	3	4
20.	Have you had difficulty in concentrating on things like reading a newspaper or watching television?	1	2	3	4
21..	Did you feel tense?	1	2	3	4
22.	Did you worry?	1	2	3	4
23.	Did you feel irritable?	1	2	3	4
24.	Did you feel depressed?	1	2	3	4
25.	Have you had difficulty remembering things?	1	2	3	4
26.	Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27.	Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28.	Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1 2 3 4 5 6 7
Very poor Excellent

30. How would you rate your overall quality of life during the past week?

1 2 3 4 5 6 7
Very poor Excellent

Appendix 4. Ethics Approval Letter

RES Committee East Midlands - Derby
Research Ethics Office
The Old Chapel Royal Standard Place
Nottingham NG1 6FS

Telephone: 0115 8839390

07 March 2014

Mr Mohamed Thaha
Senior Lecturer & Honorary Consultant in
Colorectal Surgery Queen Mary, University
of London
National Centre for Bowel Research & Surgical Innovation
1st Floor, Abernethy Building, 2 Newark Street
London
E1 2AT

Dear Mr Thaha

Study title:	**Anterior resection syndrome following sphincter-preserving surgery**
REC reference:	**14/EM/0117**
IRAS project ID:	**143889**

The Proportionate Review Sub-committee of the NRES Committee East Midlands - Derby reviewed the above application on 06 March 2014.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so.
Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Manager Ms Tracy Leavesley, NRESCommittee.EastMidlands-Derby@nhs.net.

Ethical opinion

- The sub-committee commented this is completely anonymised research.
- The sub-committee noted this is a study looking at the experiences of patients who undergo rectal resection following bowel cancer surgery.
- The sub-committee commented they view this as important research and expressed they are pleased it is now being undertaken. The sub-committee also agreed the research is valuable because it could help flag up problems participants are having following resection surgery and encourage them to speak to their GP.
- The sub-committee commented the study is essentially sound.

- The sub-committee commented the study is questionnaire based comprising of a series of questions regarding patients experiences post rectal resection.

- The sub-committee commented the target sample for this study is eight hundred people, although it is not stated how many people they expect to respond.

- The sub-committee highlighted the lack of consent form but also commented that consent is implied by completion and return of the questionnaire. The sub-committee did however comment that some participants may be confused by this.

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

1. The sub-committee would like a sentence adding to the Participant Information Sheet clarifying that consent is implied by completion and return of the questionnaire, to avoid any ambiguity or confusion.
2. The sub-committee would like clarification on the number of people the researcher envisages returning the questionnaires.

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Covering Letter	Signed by Kathryn Lynes	24 February 2014
Evidence of insurance or indemnity	Arthur J Gallagher	29 July 2013
Investigator CV	Mohamed A Thaha	13 December 2013
Investigator CV	Kathryn Lynes	17 December 2013
Letter from Sponsor	Signed by Gerry Leonard	14 February 2014
Letter of invitation to participant	1	02 December 2013
Participant Information Sheet	1	02 December 2013
Protocol	1	02 December 2013
Questionnaire	1	02 December 2013
REC application	143889/570415/1/908	21 February 2014
Referees or other scientific critique report	Signed by Bijendra Patel	07 February 2014
Referees or other scientific critique report	Mr Shafi Ahmed	09 February 2014

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website. information is available at National Research Ethics Service website > After Review

14/EM/0117	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

pp C Hallwell

Mr Peter Korczak (Chair)

Appendix 5. St Mark's Incontinence Score

	Never	Rarely	Sometimes	Weekly	Daily
Incontinence for solid stool	0	1	2	3	4
Incontinence for liquid stool	0	1	2	3	4
Incontinence for gas	0	1	2	3	4
Alteration in lifestyle	0	1	2	3	4

	No	Yes
Need to wear a pad or plug	0	2
Taking constipating medications	0	2
Lack of ability to defer defecation for 15 min (fecal urgency)	0	4

Appendix 6. Cleveland Clinic Constipation Score

Constipation Scoring System
(Agachan et al., 1996)

Name: _____ Date: _____

Frequency of bowel movements

0	1-2 times per 1-2 days
1	2 times per week
2	Once per week
3	Less than once per week
4	Less than once per month

Difficulty: painful evacuation effort

0	Never
1	Rarely
2	Sometimes
3	Usually
4	Always

Completeness: feeling incomplete evacuation

0	Never
1	Rarely
2	Sometimes
3	Usually
4	Always

Pain: abdominal pain

0	Never
1	Rarely
2	Sometimes
3	Usually
4	Always

Time: minutes in lavatory per attempt

0	Less than 5
1	5-10
2	10-20
3	20-30
4	More than 30

Assistance: type of assistance

0	Without assistance
1	Stimulative laxatives
2	Digital assistance or enema

Failure: unsuccessful attempts for evacuation per 24 hours

0	Never
1	1-3
2	3-6
3	6-9
4	More than 9

History: duration of constipation (yr)

1	0
2	1-5
3	5-10
4	10-20
5	More than 20

TOTAL SCORE: _____

(Minimum Score, 0; Maximum Score, 30)